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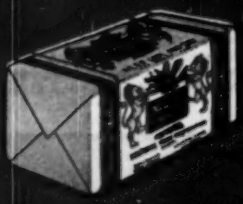

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CEREAL CHEMISTRY

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# THE INFLUENCE OF VARIOUS TEMPERATURES, HUMIDITIES, AND OXYGEN CONCENTRATIONS ON MOLD GROWTH AND BIOCHEMICAL CHANGES IN STORED YELLOW CORN[1]

## GRAIN STORAGE STUDIES IX

R. A. BOTTOMLEY[2], CLYDE M. CHRISTENSEN,[3] AND W. F. GEDDES[4]

**ABSTRACT**

The effects of variations in temperature and oxygen concentration in the atmosphere upon mold growth, viability, and several biochemical properties of No. 1 grade yellow dent corn stored for 12 days at different moisture contents, were determined in a factorially designed experiment. The moisture contents, which were in equilibrium with relative humidities of from 75 to 100%, varied from 17.4 to 31.2% (dry basis); the temperature ranged from 25° to 45°C. and the oxygen concentration varied from 21% to 0.1%.

Mold growth and the biochemical properties of the corn were affected most by the variations in relative humidity and least by the changes in atmospheric composition. The effect of each variable depended upon the levels of the others. As the relative humidity of the air in contact with the corn was increased from 75 to 100%, the total mold count increased logarithmically, the internal mold infection and fat acidity increased sharply, total and water-soluble nitrogen increased slightly, reducing sugars increased, while non-reducing sugars, total dry matter, and the viability of the grain decreased. The highest mold count was found at 25°C. and the highest fat acidity at 40°C.; the lowest values for these measures were obtained at 45°C. Lowering the oxygen content of the storage atmosphere from 21 to 0.1% decreased the extent of the various changes; the depression in mold count was four times greater than in fat acidity.

The nature of the microflora varied with moisture, temperature, and oxygen concentration. Corn in equilibrium with a relative humidity of 80%, and over all the atmospheric conditions employed, supported predominately *Penicillium* sp. at 25°C., *Aspergillus flavus* at 30°C., *A. glaucus* at

[1] Manuscript received August 24, 1949. Paper No. 2541, Scientific Journal Series, Minnesota Agricultural Experiment Station. This paper represents a portion of a thesis presented to the Graduate School of the University of Minnesota by R. A. Bottomley in partial fulfillment of the requirements for the Ph.D. degree, February, 1949.

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35°C., and *Mucor* sp. at 45°C. The tolerance of *Penicillium* sp. and *Candida pseudotropicalis* to low oxygen tensions was marked.

Total mold count and fat acidity did not show parallel trends with variations in the conditions of storage, and a low but significant correlation, $r = +0.20$, was obtained. The relation is apparently influenced by the kind of mold and the length of time of development. The highest fat acidity was found at 40°C. and coincided with the highest count of *Aspergillus flavus*, although there was a higher total mold count at 25°C.

Corn deterioration could not be estimated accurately by measurement of any one of the biochemical changes which were studied, but the decrease in non-reducing sugar content was the best single index. The values varied from zero to 174 mg. of sucrose per 10 g. of corn and bore a straight-line relation to relative humidity and to the logarithms of the mold counts for the corresponding samples.

Several workers have presented evidence which indicates that microflora are primarily responsible for the respiratory activity and deterioration of grains and oil seeds when they are stored at moisture contents above that which is in equilibrium with a relative humidity of approximately 75% (11, 14, 18, 21, 24, 25, 27, 28, 30–33, 38). On the other hand, Altschul *et al.* (1, 17, 19) contend that so far as cottonseed is concerned, the enzymic activities of the seed are of primary importance.

In addition to the water activity of the substrate, its previous history, amount of impurities present, the temperature, degree of aeration, and duration of storage influence the growth of molds (3–5, 23, 34). The degree of natural microfloral infection prior to storage has been shown to be of little importance (10).

Of the various chemical changes that occur during deterioration, the increase in fat acidity has been stressed by Zeleny (40, 41). Nagel and Semeniuk (27) demonstrated that sterilized shelled corn which was inoculated with each of nine fungi and held at about 32% moisture showed considerable increases in fat acidity. Further, other biochemical changes were noted which roughly paralleled those observed in corn which had deteriorated in storage. The use of sterilized grain in studying the role of molds in seed deterioration suffers from the fact that saprobic organisms grow on it more readily than they do on viable seeds (37) as well as from the elimination of the natural competition that exists between mold species and also from the inactivation of the enzymes of the seeds themselves.

Since attempts to preserve damp wheat and corn by storing in air-tight containers (6, 8, 20, 26) or by chemical treatment (10, 16, 22) have not proved successful, it appeared advisable to study further the conditions which govern the succession of, and competition between, fungi on stored corn together with the concomitant biochemical changes. This consideration prompted the present studies in which

the effect of variations in temperature, moisture content of the grain, and the atmospheric composition during storage upon the external and internal mold flora of the corn was determined in a factorially designed experiment. The corn, conditioned to moisture contents of 17.4, 18.8, 20.7, 23.6, 27.0, and 31.2% (dry basis) in equilibrium with relative humidities of 75, 80, 85, 90, 95, and 100%, respectively, was held at temperatures of 25, 30, 35, 40, and 45°C. under each of various atmospheres. The atmospheres ranged from air (21% oxygen) to one containing 20% carbon dioxide, 80% nitrogen and a trace of oxygen (0.1%), the oxygen being reduced and the carbon dioxide increased simultaneously in 5% increments. Several biochemical changes were followed, namely, changes in viability, fat acidity, total and water soluble nitrogen, reducing and non-reducing sugars, pH, and loss of dry matter of the stored corn.

## Materials and Methods

A composite sample of No. 1 grade yellow dent corn with 86% viability was selected. It was found to have 34% of the kernels internally infected with the molds *Fusarium* sp., *Penicillium* sp., and *Aspergillus glaucus* in approximately equal numbers. The total molds amounted to 12,000 per g.

A large water bath (5′-9″ × 1′-8″ × 1′-4″ deep with automatic temperature control and a circulating pump) was fitted with manifolds so that each gas could be delivered to, and removed from, the individual samples without using a multiplicity of connecting tubes. A continuous flow of the prepared gaseous mixtures through the humidifying solutions (12) and samples was controlled by means of a reducing valve and a screw-clamp inserted between the manifold and each of the samples. The rate of flow was measured by a calibrated flow meter.

Four prepared gaseous mixtures were obtained[5] in cylinders containing 165 cu. ft. of gas under a pressure of 2,000 lb. per sq. in. Each gas was analyzed for its oxygen and carbon dioxide content and it was found that the nitrogen-carbon dioxide mixture contained 0.38% oxygen. By passing this mixture through an acid chromous sulphate solution as described by Stone (35) and Stone and Beeson (36), the oxygen content was kept to a maximum of 0.1% and only a slight reduction was observed in the percentage of carbon dioxide present. The average analytical values for the oxygen and carbon dioxide content of the gases used in the experiment are shown below.

The laboratory compressed air that was used was passed through a cotton filter and a calcium chloride drying tower before it was humidified.

[5] Ohio Chemical and Manufacturing Company, Cleveland, Ohio.

GAS COMPOSITION BY VOLUME

| Gas | Oxygen | Carbon Dioxide | Nitrogen (by difference) |
|---|---|---|---|
| | % | % | % |
| I | 14.42 | 4.83 | 80.75 |
| II | 9.90 | 10.12 | 79.98 |
| III | 4.88 | 15.66 | 79.46 |
| IV | 0.10 | 21.33 | 78.57 |
| Air | 20.95 | 0.01 | 79.04 |

Each gas was brought to each of 75, 80, 85, 90, 95, and 99–100% relative humidity by bubbling through sulphuric acid solutions of appropriate density as determined by extrapolation of the data given by Wilson (39).

*Chemical Analyses.* Unless otherwise stated, all analyses were performed upon a sample of air-dried corn ground in a Wiley mill so as to pass a 0.5 mm. screen. To prevent contamination of one sample by another, the Wiley mill was carefully cleaned between each sample and the first portion ground was discarded.

The moisture content of the corn was determined using the 130°C., 1 hour, air-oven method described in Cereal Laboratory Methods (2). When the moisture content was above 17% (dry basis) the two-stage procedure was followed, the corn being air-dried before grinding. The moisture contents have been expressed on a dry-basis thus enabling the actual changes in water content to be observed readily.

Water-soluble nitrogen was determined by extracting a 3.0 g. sample with 100 ml. of toluol-saturated water for 16 hr. at room temperature. The extraction flasks were vigorously shaken at the beginning and at 30 min. before the end of this period, the suspension filtered and nitrogen determined in a 75.0 ml. aliquot by the Kjeldahl method (2). The total nitrogen was determined directly on the ground corn using a 1.0 g. sample.

Fat acidity was determined according to the method given in Cereal Laboratory Methods (2). Care was taken to measure the fat acidity within 2 hr. of grinding the samples, which in the interim, were kept at 4°C.

Reducing and non-reducing sugars were estimated according to the procedure given in Cereal Laboratory Methods (2) for flour analysis. The expression of the results as maltose and sucrose respectively does not infer that these are necessarily the sugars present in the corn.

The pH of the samples was determined by shaking 1.0 g. of the ground meal with 10.0 ml. of freshly redistilled water, allowing to stand for 30 min., decanting and determining the pH of this supernatant liquor with a glass electrode.

*Seed Viability, Internal Infection, and Mold Count.* The percentage viability and the extent of internal infection were determined on 50 seeds which had been surface-disinfected by washing in a 1.5% solution of sodium hypochlorite for 2 min., placed on agar in petri dishes and allowed to stand for 5 to 7 days. The percentage viability of the seed determined in this manner, agreed closely with that reported by the Minnesota State Seed Testing Laboratory.

The mold count was determined according to the method described by Christensen (9). It is essentially a measure of the number of viable spores. The writers recognize that under some circumstances fungi may grow vigorously without sporulating.

*Gas Analysis.* To ensure that the various gases were passed through the samples at a rate such that the respiration had a negligible effect upon gas composition, frequent gas analyses were necessary. Samples were taken from the effluent manifold and the carbon dioxide and oxygen content were determined with a Haldane-Henderson gas apparatus as described by Peters and Van Slyke (29).

*Experimental Procedure.* Six 900 g. lots of corn were each brought to the required moisture contents by adding the necessary amount of water, the additions being accompanied by thorough shaking of the sample. For those samples requiring more than 50 ml. of water, the additions were made in two lots with a 2 hr. interval between them. The corn was then held in closed containers for 24 hr. at 4°C.

The samples were then removed, allowed to come to room temperature, thoroughly mixed, and from each, subsamples were weighed out so as to give five lots of approximately 125 g. of dry matter. At this stage, a 10 g. sample was removed for a moisture determination as a check on the conditioning treatment.

The samples were contained in bottles of approximately 300 ml. capacity, closed with wired-in two-hole rubber stoppers each fitted with an outlet-tube and an inlet-tube reaching practically to the bottom of the sample. Immediately after connecting the sample bottles to their respective humidifying solutions (12) and to the outlet manifold, the gases were passed through at a rapid rate for 30 min. to displace all air. The rate of flow was then reduced to the minimum necessary to maintain a constant composition as determined by periodic analysis of the effluent gas.

For each temperature, 30 corn samples were tested, i.e., samples at each of six humidities for each of the five gases. The order in which the different temperatures were employed was determined by random selection.

Each trial lasted for 12 days. During this time the samples were inspected regularly and the appearance and extent of mold growth

noted. Whenever the corn appeared to be "packing," the sample bottle was shaken vigorously so that channeling of the incoming gas would be avoided so far as was possible.

At the conclusion of the trial, the samples were removed from the water-bath, transferred to tared new paper bags,[6] and weighed. Enough corn (about 35 g.) was removed for viability and internal infection tests and the weighed remainder was air-dried at room temperature for 2 to 3 days. Drying was assisted by means of a 12 in. diameter fan. After noting the air-dry weights, analyses were made as described earlier and all results were calculated on a moisture-free basis.

*Analysis of Experimental Data.* The analysis of a total of 150 samples yielded ten sets of data (mold population, germination, internal infection, fat acidity, total and water-soluble nitrogen, reducing and non-reducing sugars, pH, and loss of dry matter). These, with the exception of the results for germination and extent of internal infection, were subjected to analyses of variance (13, 15).

To study the significance of the difference between mean values for any one variable over all conditions of the other variables employed, a different error variance was used for each main effect (temperature, atmospheric composition, and relative humidity). For example, to obtain the error variance ("Emt") to test the significance of the difference due to temperature (over all humidities and atmospheres), the sums of squares for the interactions involving temperature were added to that for the second order interaction. Then, by allowing for the degrees of freedom involved, the mean square was obtained for "Emt." From this value the standard error was calculated and by applying the "t" test the difference required for significance between mean values at different temperatures was obtained at the 5% and 1% point. The value "Emt" was also used to test the significance of the main temperature effect since it was a more severe test than the use of the second order interaction. Similarly, the interactions involving atmosphere and humidity were used in conjunction with the second order interaction to calculate the error variances ("Ema" and "Emh") to be applied to the mean values for atmosphere and humidity and for the main effects, atmosphere and humidity, respectively. The second order interaction was used as error to measure the significance of the first order interactions. Several of the significant interactions have been graphed and included in the text. It should be remembered that each point in the temperature-atmosphere interaction graphs represents the mean of six values, while in the other interaction graphs each point represents the mean of five values.

[6] Tests proved that these bags were free from molds.

The co-variance of mold population and fat values was determined both for the total variation and its several components.

## Results

The analyses of variance are summarized in Table I[7] whereas the mean values for the analytical data obtained on corn stored under various temperatures, atmospheres, and relative humidities, together with the differences required for significance are given in Tables II, III, and IV, respectively.

*Mold Count.* In general, the mold count increased with increasing relative humidity and decreased as the oxygen content of the atmosphere was lowered and as the temperature was raised from 25°C. to 30°C. or higher increments. Variations in relative humidity had the greatest effect, the mold count increasing more than 300 fold with an increase in the relative humidity from 75% to 100%. The highest mold count, 121,000,000 per g., was encountered in the sample stored under air at 100% relative humidity and 25°C. There was a significant interaction between atmosphere and humidity which was mainly due to the low mold counts obtained in the absence of oxygen (Fig. 1).

TABLE I

THE EFFECTS OF VARIATIONS IN TEMPERATURE, ATMOSPHERIC COMPOSITION, AND HUMIDITY ON STORED CORN ANALYSIS OF VARIANCE

| Source of Variation | D.F. | Mean Squares | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | Molds $\times 10^{-3}$/g. | Fat Acidity[1] | Total Nitrogen | Water-sol. Nitrogen | Reducing Sugars[2] | Non-red. Sugars[3] | Loss in Dry Matter |
| | | * | ** | % ** | % ** | ** | ** | % ** |
| Temperature (*T*) | 4 | 198,009,427 * | 21,062 ** | 0.0408 ** | 0.0111 ** | 6,281 | 6,193 ** | 14.77 ** |
| Atmosphere (A) | 4 | 257,108,721 ** | 27,134 ** | 0.0021 ** | 0.0068 ** | 508 ** | 1,715 ** | 9.16 ** |
| Humidity (*H*) | 5 | 499,495,329 ** | 86,818 ** | 0.0174 * | 0.0559 * | 7,365 ** | 59,042 | 83.06 |
| $T \times A$ | 16 | 79,743,672 | 1,550 ** | 0.0008 * | 0.0011 ** | 1,057 ** | 390 ** | 0.86 ** |
| $T \times H$ | 20 | 116,348,827 ** | 6,281 ** | 0.0007 * | 0.0023 ** | 2,200 ** | 1,032 | 7.90 ** |
| $A \times H$ | 20 | 180,186,163 | 6,558 | 0.0007 | 0.0069 | 438 | 156 | 3.84 |
| Error (*E*) | 80 | 70,081,237 | 679 | 0.0040 | 0.0005 | 85 | 241 | 0.93 |
| Total | 149 | | 6,462 | 0.0022 | 0.0040 | 942 | 252 | 5.60 |
| $(T \times A)+(T \times H)+E$(=Emt) | 116 | 79,391,157 | 1,765 | 0.0005 | 0.0009 | 583 | 398 | 2.12 |
| $(A \times T)+(A \times H)+E$(=Ema) | 116 | 90,397,594 | 1,813 | 0.0005 | 0.0017 | 280 | 247 | 1.42 |
| $(H \times T)+(H \times A)+E$(=Emh) | 120 | 96,143,323 | 2,593 | 0.0005 | 0.0019 | 496 | 359 | 2.58 |

[1] Milligrams of potassium hydroxide per 100 g. corn, dry basis.
[2] As milligrams of maltose per 10 g. corn, dry basis.
[3] As milligrams of sucrose per 10 g. corn, dry basis.

[7] The pH data are not shown because the variations obtained were considered of little practical importance. The maximum range in pH encountered was from 6.6 to 5.8 units, but there were no well defined trends. The maximum difference in the mean pH values obtained with the various treatments was 0.2 units.

TABLE II

THE EFFECT OF VARIATIONS IN TEMPERATURE UPON STORED CORN, MEAN VALUES FOR ANALYTICAL DATA[1]

| Temperature | Molds $\times 10^{-3}$/g. | Fat[2] Acidity | Total Nitrogen | Water-sol. Nitrogen | Reducing Sugars[3] | Non-red. Sugars[4] | Loss in Dry Matter |
|---|---|---|---|---|---|---|---|
| °C. | | | % | % | | | % |
| 25 | 7,350 | 57.6 | 1.46 | 0.202 | 57.4 | 94.0 | 0.72 |
| 30 | 1,447 | 60.5 | 1.51 | 0.227 | 68.3 | 101.8 | 0.82 |
| 35 | 1,889 | 93.5 | 1.44 | 0.200 | 77.7 | 80.6 | 1.54 |
| 40 | 2,121 | 112.1 | 1.53 | 0.227 | 94.6 | 82.3 | 2.46 |
| 45 | 1,196 | 51.1 | 1.52 | 0.182 | 84.8 | 64.1 | 1.23 |
| Difference required for significance | | | | | | | |
| 5% Point | 4,552 | 21.5 | 0.012 | 0.015 | 12.3 | 10.2 | 0.74 |
| 1% Point | 6,028 | 27.5 | 0.016 | 0.020 | 16.3 | 13.5 | 0.99 |

[1] The values are the means for 30 samples stored at each temperature for 12 days; that is, for samples stored at each of six relative humidities (75 to 100%) and five atmospheres (0.1 to 21% oxygen).
[2] Milligrams of potassium hydroxide per 100 g. corn, dry basis.
[3] As milligrams of maltose per 10 g. corn, dry basis.
[4] As milligrams of sucrose per 10 g. corn, dry basis.

The times taken for mold growth to become visible to the naked eye are shown in Table V. Samples stored under the least favorable conditions for mold growth showed no visible molds at 12 days when the test was concluded, yet mold assays in many instances gave appreciable counts; for example, samples stored at 45°C. and 75 to 85% relative humidity, which appeared bright and normal, gave mold counts up to 218,000 per gram.

TABLE III

THE EFFECT OF VARIATIONS IN ATMOSPHERIC COMPOSITION UPON STORED CORN, MEAN VALUES FOR ANALYTICAL DATA[1]

| Atmosphere Oxygen | Molds $\times 10^{-3}$/g. | Fat[2] Acidity | Total Nitrogen | Water-sol. Nitrogen | Reducing Sugars[3] | Non-red. Sugars[4] | Loss in Dry Matter |
|---|---|---|---|---|---|---|---|
| % | | | % | % | | | % |
| 21 | 7,619 | 81.9 | 1.50 | 0.226 | 78.3 | 80.1 | 2.04 |
| 15 | 3,221 | 98.4 | 1.50 | 0.211 | 81.1 | 79.3 | 1.43 |
| 10 | 1,982 | 88.1 | 1.49 | 0.216 | 73.9 | 82.7 | 1.56 |
| 5 | 1,117 | 84.0 | 1.46 | 0.199 | 70.9 | 82.9 | 1.16 |
| 0 | 65 | 22.4 | 1.48 | 0.187 | 78.9 | 97.8 | 0.54 |
| Difference required for significance | | | | | | | |
| 5% point | 4,861 | 21.8 | 0.012 | 0.021 | 8.5 | 8.0 | 0.61 |
| 1% point | 6,432 | 28.9 | 0.016 | 0.028 | 11.3 | 10.6 | 0.81 |

[1] The values are the means for 30 samples stored under each atmosphere for 12 days; that is, for samples stored at each of five temperatures (25°–45°C.) and six relative humidities (75 to 100%).
[2] Milligrams of potassium hydroxide per 100 g. corn, dry basis.
[3] As milligrams of maltose per 10 g. corn, dry basis.
[4] As milligrams of sucrose per 10 g. corn, dry basis.

TABLE IV

THE EFFECT OF VARIATIONS IN RELATIVE HUMIDITY UPON STORED CORN, MEAN VALUES FOR ANALYTICAL DATA[1]

| Relative Humidity | Molds $\times 10^{-3}$/g. | Fat Acidity[2] | Total Nitrogen | Water-sol. Nitrogen | Reducing Sugars[3] | Non-red. Sugars[4] | Loss in Dry Matter |
|---|---|---|---|---|---|---|---|
| % | | | % | % | | | % |
| 75 | 36 | 23.4 | 1.47 | 0.180 | 58.4 | 143.0 | 0.28 |
| 80 | 59 | 24.1 | 1.47 | 0.180 | 68.4 | 127.0 | 0.30 |
| 85 | 409 | 35.6 | 1.48 | 0.180 | 74.1 | 104.6 | 0.32 |
| 90 | 1,008 | 71.4 | 1.49 | 0.188 | 72.9 | 71.7 | 0.72 |
| 95 | 3,843 | 143.6 | 1.50 | 0.217 | 77.0 | 39.6 | 1.58 |
| 100 | 11,445 | 151.6 | 1.54 | 0.300 | 109.0 | 16.3 | 4.93 |
| Difference required for significance | | | | | | | |
| 5% point | 5,491 | 26.1 | 0.013 | 0.024 | 12.5 | 10.6 | 0.90 |
| 1% point | 7,266 | 34.5 | 0.017 | 0.032 | 16.5 | 14.0 | 1.19 |

[1] The values are the means for 25 samples stored at each relative humidity for 12 days; that is, for samples stored at each of five temperatures (25 to 45°C.) and under five atmospheres (0.1% to 21% oxygen).
[2] Milligrams of potassium hydroxide per 100 g. corn, dry basis.
[3] As milligrams of maltose per 10 g. corn, dry basis.
[4] As milligrams of sucrose per 10 g. corn, dry basis.

The predominant flora noted during the mold assays at the end of the 12-day storage period are reported for each corn sample in Table VI. No attempt was made to identify the various species of *Penicillium* and *Mucor* that occurred and only one mold has been recorded if it

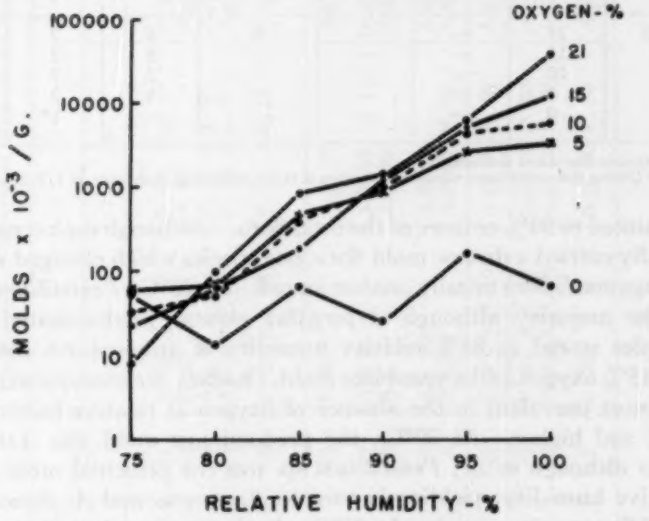


FIG. 1. The effect of atmosphere and relative humidity on the mean mold count (plotted on a logarithmic scale) of corn stored for 12 days at five temperatures (25–45°C.).

TABLE V

TIME IN DAYS TO FIRST VISIBLE MOLD GROWTH

| Temperature | Atmos. | Relative Humidity % | | | | | |
|---|---|---|---|---|---|---|---|
| | | 75 | 80 | 85 | 90 | 95 | 100 |
| | | First visible mold growth—days | | | | | |
| °C. | $O_2$% | | | | | | |
| 25 | 21 | — | 5 | 4 | 4 | 3 | 3 |
| | 15 | — | 9 | 6 | 4 | 3 | 3 |
| | 10 | — | 9 | 6 | 4 | 3 | 3 |
| | 5 | — | — | 7 | 6 | 3 | 3 |
| | 0 | — | — | 10 | — | 10 | 10 |
| 30 | 21 | — | 9 | 8 | 3 | 3 | 3 |
| | 15 | — | — | 8 | 3 | 3 | 3 |
| | 10 | — | — | 9 | 3 | 3 | 3 |
| | 5 | — | — | 9 | 8 | 4 | 3 |
| | 0 | — | — | — | — | — | — |
| 35 | 21 | — | 4 | 3 | 3 | 3 | 3 |
| | 15 | 4 | 4 | 3 | 3 | 3 | 3 |
| | 10 | — | 9 | 4 | 3 | 3 | 3 |
| | 5 | — | 9 | 4 | 3 | 3 | 3 |
| | 0 | — | — | 10 | 3 | 3 | 3 |
| 40** | 21 | — | — | 7 | 3 | 3 | 3 |
| | 15 | — | 7–12 | 7–12 | 3–7 | 3 | 3 |
| | 10 | — | — | 7–12 | 3–7 | 3 | 3 |
| | 5 | — | — | 7–12 | 3–7 | 3 | 3 |
| | 0 | — | — | — | — | 1* | 1* |
| 45 | 21 | — | — | 9 | 6 | 2 | 1 |
| | 15 | — | — | — | 6 | 2 | 2 |
| | 10 | — | — | — | 2 | 2 | 2 |
| | 5 | — | — | — | 6 | 2 | 2 |
| | 0 | — | — | — | — | 1* | 1* |

* Grains discolored and sour.
** During this experiment visual examination of the samples was made only on 1, 3, 7, and 12 days.

amounted to 90% or more of the total flora. Although each corn sample usually carried a diverse mold flora, one species which changed with the storage conditions usually predominated. At 25°C., *Penicillium* sp. was in the majority although *Aspergillus glaucus* predominated in the samples stored at 85% relative humidity in atmospheres containing 5 to 15% oxygen and a yeast-like mold, *Candida pseudotropicalis* sp. was the most prevalent in the absence of oxygen at relative humidities of 90% and higher. At 30°C., the predominant mold was *Aspergillus flavus* although in air, *Penicillium* sp. was the principal mold at 75% relative humidity, yielding in turn to *A. glaucus* and *A. flavus* as the humidity was increased. At 35°C., *A. glaucus* was the principal mold in samples stored at 80 to 90% relative humidity while *A. flavus* predominated at higher humidities. At 40°C., *A. flavus* flourished at both

TABLE VI

PREDOMINANT MOLDS AT END OF 12-DAY STORAGE PERIOD AS PER CENT OF TOTAL MOLDS

| Temp. | Atmos. $O_2$ | Relative Humidity—% | | | | | |
|---|---|---|---|---|---|---|---|
| | | 75 | 80 | 85 | 90 | 95 | 100 |
| °C. | % | | | | | | |
| 25 | 21 | P. 60, A.gl. 30 | P. 90, A.gl. 10 | P. 45, A.gl. 45 | P. 50, A.gl. 40 | P. 70, A.fl. 20 | P. 90, A.fl. 10 |
| | 15 | P. 92 | P. 90 | A.gl. 90 | P. 60, A.gl. 30 | P. 90 | P. 80, A.fl. 20 |
| | 10 | A.fl. 99 | P. 50, A.fl. 50 | A.gl. 99 | P. 55, A.gl. 40 | P. 90, A.gl. 10 | P. 90, A.fl. 10 |
| | 5 | A.fl. 40, P. 40, M. 20 | P. 35, A.fl. 35 | A.gl. 90 | P. 50, A.gl. 45 | P. 95 | P. 90, A.fl. 10 |
| | 0 | P. 70, A.fl. 30 | P. 55, A.fl. 45 | P. 99 | Can. 70, P. 15, A.gl. 10 | Can. 99 | P. 65, Can. 30 |
| 30 | 21 | P. 90 | A.gl. 99 | A.gl. 99 | A.fl. 60, P. 25, A.gl. 10 | A.fl. 75 | A.fl. 95 |
| | 15 | A.fl. 99 | A.fl. 99 | A.fl. 99 | A.fl. 99 | A.fl. 99 | A.fl. 99 |
| | 10 | A.fl. 99 | A.fl. 90 | A.fl. 45, A.gl. 55 | A.fl. 85, A.gl. 15 | A.fl. 80 (A.gl., P., M.) | A.fl. 99 |
| | 5 | A.fl. 99 | A.fl. 99 | A.fl. 99 | A.fl. 99 | A.fl. 99 | A.fl. 99 |
| | 0 | A.fl. 90 | A.fl. 90 (P., M.) | A.fl. 90 (P., M.) | A.fl. 85 (P., M.) | A.fl. 85 (P., M.) | A.fl. 75, A.gl. 25 |
| 35 | 21 | P. 80, A.gl. 10 | A.gl. 80, P. 20 | A.gl. 99 | A.gl. 85, A. och. 5 | A.fl. 90 (A.gl., M.) | A.fl. 99 |
| | 15 | P. 50, A.gl. 50 | A.gl. 99 | A.gl. 99 | A.gl. 99 | A.gl. 99 | A.fl. 99 |
| | 10 | P. 35, A.gl. 60 | A.gl. 70, P. 30 | A.gl. 99 | A.gl. 90 | A.gl. 65, A.fl. 30 | A.fl. 99 |
| | 5 | P. 65, A.gl. 30 | A.gl. 90, P. 10 | A.gl. 99 | A.gl. 80, A.fl. 15 (M.) | A.gl. 30, A.fl. 60 (M.) | A.fl. 99 |
| | 0 | P. 99 | A.gl. 50, P. 50 | A.gl. 33, P. 67 | A.gl. 75, A.fl. 25 | A.gl. 20, A.fl. 80 | A.fl. 99 |

TABLE VI—*Continued*

| Temp. | Atmos. $O_2$ | Relative Humidity—% | | | | | |
|---|---|---|---|---|---|---|---|
| | | 75 | 80 | 85 | 90 | 95 | 100 |
| °C. | % | | | | | | |
| 40 | 21 | A.fl. 99 | A.gl. 90, A.fl. 10 | A.gl. 99 | A. gl.90 (A.fl., M.) | A.fl. 99 (M.) | A.fl. 99 (M.) |
| | 15 | A.fl. 99 (M.) | A.gl. 99 | A.gl. 99 | A.gl. 99 | A.fl. 99 (M.) | A.fl. 99 (M.) |
| | 10 | A.fl. 20, P. 75 | A.gl. 25, P. 75 | A.gl. 99 | A.gl. 95 | A.fl. 85 | A.fl. 90 |
| | 5 | A.fl. 90 (P., M.) | A.gl. 95 | A.gl. 95 | A.gl. 75, A.fl. 15 | A.fl. 75, M. 20, A.n. 5, | A.fl. 80, M. 15 |
| | 0 | A.fl. 75 (M.) | A.gl. 70, A.fl. 25 | A.gl. 90 (A.fl., A.n.) | A.gl. 85 (A.fl., M.) | A.fl. 65, M. 25 (A.gl.) | A.fl. 80, A.n. 10 (M.) |
| 45 | 21 | A.fl. 40, P. 60 | P. 40, M. 40, A.fl. 20 | A.ter. 45, M. 30 (P., A.gl.) | M. 50, P. 50 | M. 75, P. 20 | P. 80, M. 20 |
| | 15 | A.fl. 20, P. 75 | P. 85, A.fl. 15 | P. 50, A.gl. 50 | M. 95, A. ter. 5 | M. 90 | P. 85, A.gl. 10 |
| | 10 | M. 60, P. 40 | M. 60, A.fl. 20, P. 20 | M. 70, P. 30 | M. 85 (P., Asp.) | M. 99 | M. 55, P. 45 |
| | 5 | M. 40, A.gl. 40, P. 10 | M. 45, P. 35 | M. 45, P. 45, A.fl. 10 | M. 75, A.ter. 15 | M. 90, P. 10 | P. 99 |
| | 0 | M. 35, P. 55, A.fl. 15 | M. 25, P. 50, A.fl. 15 | M. 35, P. 35, A.fl. 20 | M. 15, P. 45, A.fl. 35 | M. 25, P. 55 | M. 40, P. 45, Asp. 15 |

Abbreviations: P.—*Penicillium* sp.
A.gl.—*Aspergillus glaucus*
A.fl.—*A. flavus*
A.ter.—*A. terreus*
A.och.—*A. ochraceus*
A.n.—*A. niger*
Asp.—Unidentified *Aspergillus* sp.
M.—*Mucor* sp.
Can.—*Candida* sp.

low and high humidities, but *A. glaucus* was dominant at 80 to 90% relative humidity, inclusive. At 45°C., the incidence of *A. flavus* and *A. glaucus* was considerably reduced, *Mucor* sp. and *Penicillium* sp. taking their place.

It must be emphasized that the mold studies were made at the end of the 12-day storage period. The relative preponderance of the various species probably changed throughout the trial and mold assays made only at the end of the period may be of limited value in estimating the numbers and kinds of molds in relation to the deterioration which has occurred in the grain.

*Internal Infection of the Corn.* The effects of variations in temperature, atmosphere, and relative humidity on the percentage of corn kernels which were internally infected with molds are shown in Table VII. The nature of the predominant internal flora is recorded in

TABLE VII

PERCENTAGE OF CORN KERNELS SHOWING INTERNAL INFECTION WITH MOLDS FOLLOWING STORAGE FOR 12 DAYS UNDER VARIOUS CONDITIONS[1]

| | | | | | | |
|---|---|---|---|---|---|---|
| Temperature, °C. | 25 | 30 | 35 | 40 | 45 | |
| Mean internal infection, % | 36.1 | 40.7 | 38.7 | 36.7 | 35.0 | |
| Atmosphere—oxygen, % | 21 | 15 | 10 | 5 | 0 | |
| Mean internal infection, % | 46.3 | 41.9 | 37.7 | 42.8 | 17.7 | |
| Relative humidity, % | 75 | 80 | 85 | 90 | 95 | 100 |
| Mean internal infection, % | 1.8 | 4.9 | 14.6 | 35.2 | 81.7 | 86.3 |

[1] The values for internal infection recorded for each temperature, atmosphere, and relative humidity, respectively, are means for all conditions of the other two variables. For example, the data for each temperature are the means for 30 samples (five atmospheres at each of six relative humidities).

Table VIII. The results approximately parallel those obtained for total mold count.

*Corn Viability.* The viability of the corn was adversely affected by all storage conditions, but variations in oxygen concentration had less effect that increases in temperature or relative humidity (Table IX). Few samples stored at 100% relative humidity or at 45°C. were viable. The least reduction in germination occurred in the sample stored at 30°C. under 75% relative humidity and "zero" oxygen content for which a viability of 60% (a decrease of 26%) was recorded.

*Fat Acidity.* Variations in the relative humidity of the storage atmosphere had a greater effect on fat acidity formation than differences in temperature or oxygen concentration (Tables II, III, and IV). Fat acidity increased from a minimum value of 23.4 to a maximum of 151.6 as the relative humidity increased from 75 to 100% (Table II). The mean value obtained at 100% relative humidity was only a little greater than that found at 95%. In some instances a fat acidity value

TABLE VIII

THE PREDOMINANT MOLDS COMPRISING THE INTERNAL INFECTION OF CORN FOR 12 DAYS

| Temp. | Atmos. $O_2$ | Relative Humidity—% | | | | | |
|---|---|---|---|---|---|---|---|
| | | 75 | 80 | 85 | 90 | 95 | 100 |
| °C. | % | | | | | | |
| 25 | 21 | F. | — | F., P., A.gl., M. | P., A.fl. | P., M., A.fl., Alt. | M., P. |
| | 15 | P. | F. | F., M., Alt. | P., A.fl., F. | P., F., A.fl. | M., A.fl., P. |
| | 10 | — | F., Alt. | Can., P. | P., A.fl. | P., A.fl., M. | M., A.fl., P. |
| | 5 | F. | F., P. | F., P. | P., A.fl., F. | A.fl., P., M. | F., A.fl., M. |
| | 0 | F. | F., P. | — | M., F. | F., M. | F. |
| 30 | 21 | — | A.gl. | Asp., F. | A.fl., P. | P., A.fl., M., F. | F., M., A.fl., A.n. |
| | 15 | — | Can. | P. | A.fl., P., F. | A.fl., P., F. | A.fl., P., F., M. |
| | 10 | — | F., Can. | F., Can., P. | A.fl., P. | A.fl., F., P., M. | F., M., A.fl. |
| | 5 | P. | — | Can., P. | A.fl., P. | F., A.fl., P. | F., A.fl. |
| | 0 | — | P., M. | — | Can. | Can., F., M. | F., A.fl. |
| 35 | 21 | F. | A.fl., P., F. | P., Asp. | Can., Asp., M., F. | A.fl., M., F. | A.fl., M., F. |
| | 15 | P., F. | M., P., A.gl. | Can., P., M., F. | A.fl., P., M. | A.fl., F. | A.fl., M., F., P. |
| | 10 | — | P., M. | P., M., Alt. | A.fl., M. | A.fl., M., F. | A.fl., M., F., P. |
| | 5 | P., M. | P., M., F. | P., M., F. | A.fl., A.och. | A.fl., F., M., Asp. | F., A.fl. |
| | 0 | F. | Can. | P., F. | A.fl., F., A.n. | A.fl., F., M. | F., A.fl., M. |
| 40 | 21 | — | A.fl., F. | A.gl., F. | A.fl., A.gl. | A.fl., M. | A.fl. |
| | 15 | — | A.gl., A.fl. | A.gl. | A.gl., A.fl., Can. | A.fl., A.och., Can. | A.fl., M., P. |
| | 10 | — | — | A.gl., Can. | A.fl., A.gl., M. | A.gl., M. | A.fl., M., A.n. |
| | 5 | — | A.gl. | A.gl. | A.fl., A.gl., M. | A.fl., M., A.och. | A.fl., M., P. |
| | 0 | — | M. | — | Can. | Can. | Can. |
| 45 | 21 | — | — | Can. | M., Can., A. fum. | A.fum., M. | A.fum., M., Can. |
| | 15 | — | — | — | A.fum., Can., M. | A.fum., Can. | A.fum., Can. |
| | 10 | — | — | — | A.fum., M. | A.fum., M. | A.fum., M. |
| | 5 | Can. | — | Can. | A.fum., Can. | A.fum. | A.fum. |
| | 0 | A.fl. | — | Can. | Can. | Can. | Can. |

Abbreviations: F.—*Fusarium* M.—*Mucor* sp. Can.—*Candida* sp. A. gl.—*A. glaucus* A.och.—*A. ochraceus* Asp.—Unidentified
Alt.—*Alternaria* P.—*Penicillium* sp. A.fl.—*Aspergillus flavus* A.fum.—*A. fumigatus* A.n.—*A. niger* *Aspergillus* sp.

TABLE IX

THE VIABILITY OF CORN FOLLOWING STORAGE FOR 12 DAYS UNDER VARIOUS CONDITIONS[1]

| | | | | | | |
|---|---|---|---|---|---|---|
| Temperature, °C. | 25 | 30 | 35 | 40 | 45 | |
| Mean viability, % | 20.1 | 18.9 | 9.8 | 6.0 | 0.3 | |
| Atmosphere—oxygen, % | 21 | 15 | 10 | 5 | 0 | |
| Mean viability, % | 7.8 | 9.8 | 10.4 | 11.4 | 14.6 | |
| Relative humidity, % | 75 | 80 | 85 | 90 | 95 | 100 |
| Mean viability, % | 27.0 | 18.4 | 11.3 | 6.8 | 2.2 | 0.6 |

[1] The viability values recorded for each temperature, atmosphere, and relative humidity, respectively, are means for all conditions of the other two variables. For example, the data for each temperature are the mean viabilities for 30 samples (five atmospheres at each of six relative humidities).

The control sample showed 86% germination and only fell to 82.5% after several months storage at 4°C.

was noted which was lower at 100% than at 95% relative humidity, although the samples had been held under the same conditions of temperature and atmosphere.

As the temperature increased from 25° to 40°C., there was a rise in fat acidity from 57.6 to 112.1, followed by a decrease to 51.1 at 45°C.

Decreasing the oxygen content from 21 to 5% produced no significant changes in mean fat acidity values. However, with 0.1% oxygen there was a highly significant reduction in fat acidity as compared with any of the other values.

The interactions of temperature × atmosphere, temperature × humidity, and atmosphere × humidity on fat acidity are illustrated

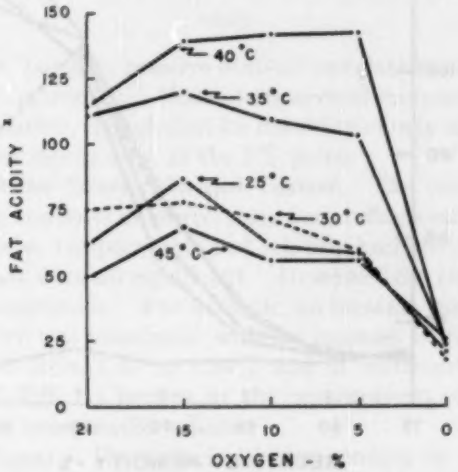


FIG. 2. The effect of temperature and atmosphere on the mean fat acidity of corn stored for 12 days at six relative humidities (75–100%). Fat acidity is expressed as mg. of potassium hydroxide per 100 g. of corn (dry basis).

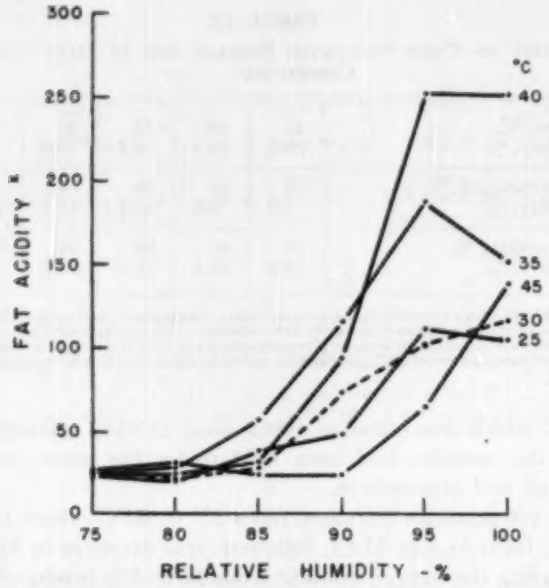


FIG. 3. The effect of temperature and relative humidity on the mean fat acidity of corn stored for 12 days under five atmospheres (0.1 to 21% oxygen). Fat acidity is expressed as mg. of potassium hydroxide per 100 g. of corn (dry basis).

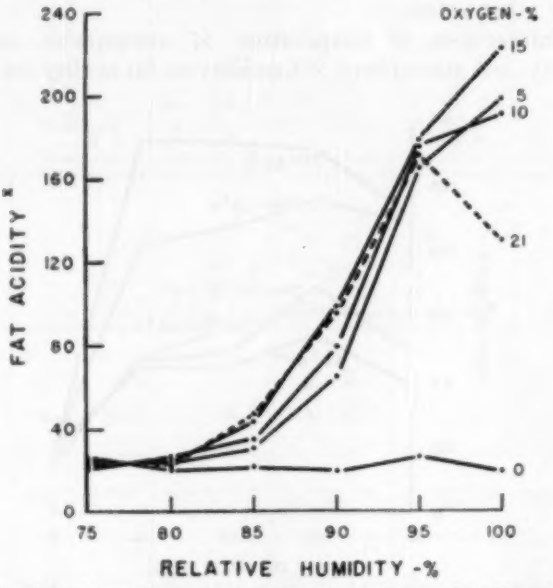


FIG. 4. The effect of atmosphere and relative humidity on the mean fat acidity of corn stored for 12 days at five temperatures (25–45°C.). Fat acidity is expressed as mg. of potassium hydroxide per 100 g. of corn (dry basis).

in Figs. 2, 3, and 4. The interaction of temperature × atmosphere is largely due to the fact that at 40°C., the maximum acidity was recorded in 5% oxygen while for all other temperatures the maxima were reached in 15% oxygen.

The interaction of temperature × humidity upon fat acidity is due largely to the much greater increases in fat acidity with increases in humidity from 80 to 95% at 35° and 40°C. than at other temperatures. A marked decrease in fat acidity with increase in humidity from 95 to 100% was noted at 35°C.

The anomalies in the curves from 25°C. shown in Figs. 2 and 3 may be associated with the large populations of *Penicillium* which predominated only at this temperature.

Figure 4 shows that fat acidity was uninfluenced by increasing the relative humidity unless oxygen was present. In the presence of various concentrations of oxygen, the increases in fat acidity with increasing humidity were quite uniform except that in air (21% oxygen), the maximum value was recorded at 95% relative humidity.

The correlations between the mold counts and fat acidity values obtained by an analyses of covariance are as follows:

| *Component* | *r* | *Expected r at 5% point* |
|---|---|---|
| Temperature (*T*) | −0.23 | 0.95 |
| Atmosphere (*A*) | +0.46 | 0.95 |
| Humidity (*H*) | +0.85 | 0.88 |
| $T \times A$ | −0.03 | 0.49 |
| $T \times H$ | −0.26 | 0.44 |
| $A \times H$ | +0.01 | 0.44 |
| Error | +0.05 | 0.22 |
| Total | +0.20 | 0.16 |

A significant, but low, positive over-all correlation existed between mold count and fat acidity. None of the several components showed a significant correlation, though that for humidity closely approached the level required for significance at the 5% point.

*Total and Water-Soluble Nitrogen Content.* The total and water-soluble nitrogen contents of stored corn were influenced by variations in the atmosphere, temperature, and relative humidity and the first order interactions were all significant. However, the changes were of a low order of magnitude. For example, an increase from 75 to 100% relative humidity was associated with an increase in the mean total nitrogen content from 1.47 to 1.54% and in water-soluble nitrogen from 0.18 to 0.30%. Changes in the atmospheric composition or temperature had even smaller effects.

*Reducing Sugars.* The reducing sugar content of the corn was markedly influenced by variations in the temperature and relative humidity of the storage atmosphere but not by changes in atmospheric

composition (Table I). The maltose values increased with increases in temperature between 25° to 40°C. and also as the humidity was raised, the maximum value being obtained for 100% relative humidity (Tables II, IV).

The first order interactions were all highly significant. That for temperature and atmosphere was derived largely from the abnormally high reducing sugar content of corn stored under 15% oxygen at 40°C. and of the corn stored under a nearly oxygen-free atmosphere at 45°C. These results are not entirely in harmony with the mold data and the question arises whether the reducing sugar values are a good index of the total metabolic activity of a changing mold flora.

The interaction of temperature × humidity was due principally to the abnormally high reducing sugar values for the corn stored at 100% relative humidity at temperatures of 35° and 40°C. As *Aspergillus flavus* was the predominant mold present under these conditions, these high maltose values were associated with the presence of a mold known to form α-amylase (7) and with conditions which favor its activity.

The interaction of atmosphere × humidity upon the reducing sugar values of the stored corn was due largely to greater increases in the reducing sugar content of the samples stored under 15 and 21% oxygen when the relative humidity was increased from 95 to 100% than was the case for the samples stored under lower oxygen levels.

*Non-Reducing Sugars.* High values for non-reducing sugars indicate the presence of low enzyme activity and vice versa. This is the opposite of the case with the reducing sugars which have just been discussed. The maximum disappearance of non-reducing sugars at different temperatures occurred at 45°C. at which the lowest mean mold population was found. There is the possibility that the *Mucor* noted at this temperature was more active in secreting invertase than the species of molds which were predominant at lower temperatures. On the other hand, the low non-reducing sugar content may be related to bacterial activity at the higher relative humidities or to an increase in invertase activity with increase in temperature. Further investigation is necessary to provide an interpretation of these results.

Variation in atmosphere had little effect upon the quantity of non-reducing sugars found in the stored corn except when the oxygen content was lowered to 0% (Table III). At this level, the non-reducing sugars significantly exceeded the amounts at all of the other concentrations (Table III). The major difference between atmospheres in their effect upon the disappearance of non-reducing sugars occurred at 25°C. when the range in mean values was from 77.8 to 128.2 mg. per 10 g. of corn.

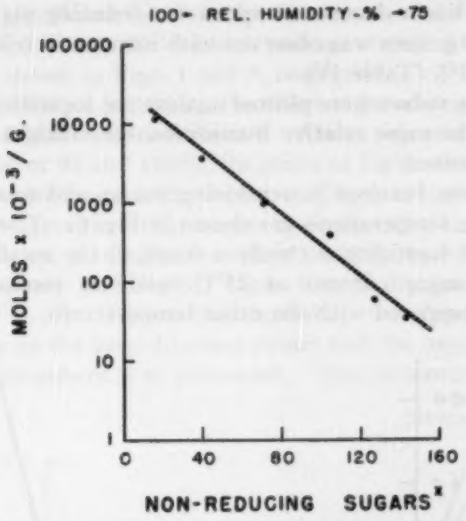


FIG. 5. The relation between mold count (plotted on a logarithmic scale) and non-reducing sugar content of corn stored under relative humidities of from 75 to 100%. Each point represents the mean of 25 values; that is, for corn samples stored at each of five temperatures (25–45°C.) and five atmospheres (0–21% oxygen). Non-reducing sugars are expressed as mg. sucrose/10 g. corn, dry basis.

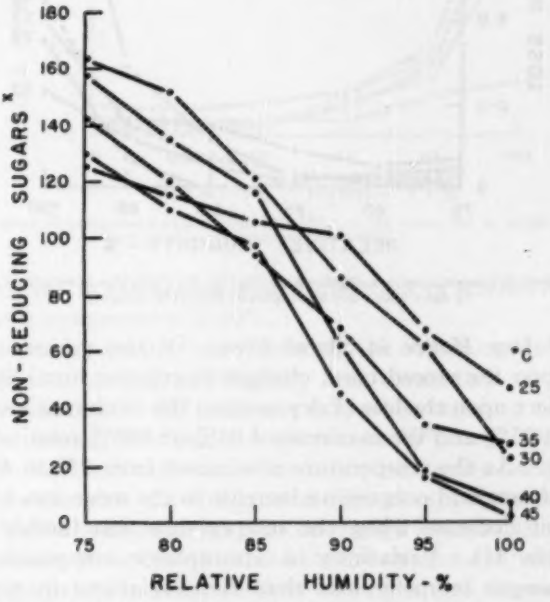


FIG. 6. The effect of temperature and relative humidity on the mean non-reducing sugar content of corn stored 12 days under five atmospheres (0.1–21% oxygen). Non-reducing sugars are expressed as mg. sucrose/10 g. corn, dry basis.

An almost linear decrease in mean non-reducing sugars from 143.0 to 16.3 mg./10 g. corn was observed with increase in relative humidity from 75 to 100% (Table IV).

When these values were plotted against the logarithms of the mold contents for the same relative humidities, the straight line shown in Fig. 5 was obtained.

The relations between non-reducing sugars and relative humidity for the various temperatures are shown in Fig. 6. The interaction of temperature × humidity is chiefly a result of the smaller decrease in non-reducing sugar content at 25°C. with an increase in relative humidity as compared with the other temperatures.

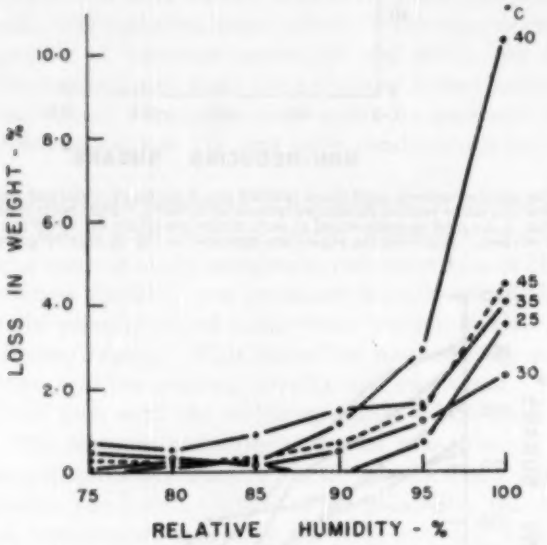


FIG. 7. The effect of temperature and relative humidity on the mean loss in weight of corn stored for 12 days under five atmospheres (0.1–21% oxygen).

*Loss of Dry Matter in Stored Corn.* Of the various conditions imposed upon the stored corn, changes in relative humidity had the greatest effect upon the loss of dry matter; the minimum loss of 0.28% occurred at 75% and the maximum 4.93% at 100% relative humidity (Table IV). As the temperature was raised from 25° to 40°C. there was a significant and progressive increase in the mean loss followed by a significant decrease when the temperature was further raised to 45°C (Table II). Variations in atmospheric composition caused smaller changes in mean loss than did variations in temperature (Table II). The maximum loss occurred when the corn was stored under air and the minimum under anaerobic conditions.

The highly significant relationships between loss of dry matter and relative humidity over the various storage temperatures and atmospheres are shown in Figs. 7 and 8, respectively.

Below 90% relative humidity, the effect of increasing the temperature from 25° to 45°C. was not marked. Above this relative humidity, and particularly at 95 and 100%, the effect of higher temperature was reflected by greater losses of dry matter. The loss sustained at 40°C. was very much larger than that occurring at the other temperatures.

Similarly, below a relative humidity of 90% the effects of variations in atmosphere or humidity upon loss of dry matter were not pronounced (Fig. 8). Above this humidity the dry matter losses became greater as the humidity was raised and the oxygen content of the storage atmosphere was increased. The maximum loss in dry

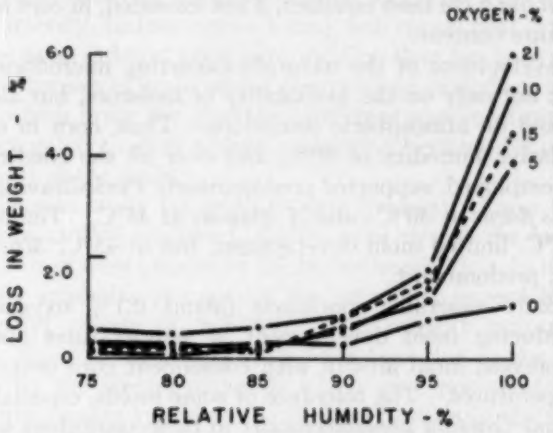


FIG. 8. The effect of atmosphere and relative humidity on the mean loss in weight of corn stored 12 days at five temperatures (25 to 45°C.).

matter occurred in the sample stored under air of a relative humidity of 100% at a temperature of 40°C.

The loss in dry matter experienced in the stored corn is a result of seed respiration and/or metabolism and respiration of the molds. Actual germination of the corn was only observed in a few seeds of one sample, namely that stored under air at 30°C. and 100% relative humidity. Seed respiration could not account for the losses noted (up to 2.5%) in samples stored anaerobically, since Milner and Geddes (23) have shown seed respiration to be negligible under this condition. While increases in temperature and in the oxygen content of the atmosphere tended to increase the loss in weight and the mold population, a plot of the data indicated that there was little correlation between the two factors. This is not surprising in view of the differences

reported by Nagel and Semeniuk (27) in the ability of various molds to decompose organic matter. However, the maximum mean loss in dry matter did occur at 40°C., the temperature which supported the maximum growth of *Aspergillus flavus*, a mold known to be highly active in decomposing corn.

## Discussion

This factorial experiment was designed to give the naturally-occurring microflora on No. 1 grade yellow corn an unhindered opportunity for development under conditions of temperature and atmosphere which could occur during commercial storage. To evaluate the role of molds in the deterioration of the grain, levels of moisture were employed which were above those permitted in No. 1 grade corn. It may safely be assumed that the deterioration which followed would have been equalled, if not exceeded, in corn of naturally high moisture content.

The development of the naturally-occurring microflora of corn is dependent not only on the availability of moisture, but also on temperature and on atmospheric conditions. Thus, corn in equilibrium with a relative humidity of 80%, and over all the conditions of atmosphere employed, supported predominately *Penicillium* sp. at 25°C., *Aspergillus flavus* at 30°C., and *A. glaucus* at 35°C. Temperatures of 40 and 45°C. limited mold development, but at 45°C. *Mucor* sp. was, in general, predominant.

Essentially anaerobic conditions (about 0.1% oxygen), though greatly reducing mold development at temperatures above 25°C., failed to prevent mold growth with consequent corn deterioration at these temperatures. The tolerance of some molds, especially *Penicillium* sp. and *Candida pseudotropicalis* to these conditions was marked and indicates the impracticability of storing high-moisture grain under hermetically-sealed, anaerobic conditions.

Although a significant, positive correlation ($r = +0.20$) was obtained between mold count and fat acidity, it was too low to be of practical value. Reports in the literature, including previous studies from these laboratories, have almost invariably shown that factors which favor an increase in mold population also result in a rather parallel increase in fat acidity. In fact, fat acidity has been regarded as the most valuable single index of the "commercial condition" of grain (42). The question naturally arises whether the poor correlation obtained in the present study represents the true existing relation or whether it is the result of failure of the experimental techniques to provide a reliable measure of the numbers or metabolic activities of the molds under the widely varying experimental conditions to which the corn was subjected.

The plates made to determine the extent of mold contamination actually only yield counts of the mold spores which germinated to produce individual colonies and of fragments of mycelium which produced individual colonies. Although the number of viable spores may serve as a rough criterion of the extent of mold growth as a whole, it is well-known that with some molds certain conditions which favor mycelial development do not favor sporulation. Moreover, the mold assays were made at the end of the 12-day trial and complex ecological changes in the mold flora probably occurred under some of the storage conditions. Different species of molds are known to differ greatly in the amounts of lipase and other enzymes which they produce and in their ability to metabolize such products as fatty acids and maltose. Although limitations in the methods of measuring mold activity may be in part responsible for the poor relation found between mold count and lipase activity, further research may well reveal that fat acidity is of less value as an index of grain deterioration than has been commonly susposed. Samples of grain stored over various periods of time under conditions which favor the growth of different species of molds would hardly be expected to show a close association between fat acidity and the soundness of the grain.

In the present study fat acidities were frequently lower in samples at 100% than at 95% relative humidity even though the mold counts and deterioration were greater at the higher humidity. In no instance of essentially anaerobic storage did the fat acidity exceed 40 notwithstanding mold counts of 765,000/g. and evident unsoundness of the grain. The low fat acidity may have been due to the low lipase production by the species of molds which were prevalent under the extremely low oxygen tension or to their utilization of the fatty acids which were produced. Further studies under controlled conditions of the metabolic activity of the principal molds involved in the deterioration of stored seeds are needed.

The variations in the total nitrogen content of the stored corn are thought to be due to the removal of carbohydrates through seed or mold metabolism, the nitrogen content of the remainder increasing with increase in loss of dry matter. The trend in mean total nitrogen content for the different humidities used in the experiment parallels that for the loss in dry matter, both increasing as the humidity was raised.

The greatest changes in the content of water-soluble nitrogen were observed at 100% relative humidity at all temperatures. While it is suspected that alterations in this factor reflect the proteolytic activity of the various molds there are some indications that factors other than mold growth may be involved.

The relatively slight changes occurring in the pH values demonstrate that this determination is of little value in the estimation of the soundness of corn. The buffer capacity of corn is sufficiently high to prevent drastic changes in acidity.

The estimation of the non-reducing sugar content was the best single index for determining the extent of deterioration which the corn suffered. In no instance was a low non-reducing sugar content found when the corn was but little damaged, as judged from all the other factors measured. The range in values was 174 to 0 mg. of sucrose per 10 g. of corn, which is sufficiently large to permit clear differentiation between various samples. The mean sucrose values showed an almost linear decrease with increase in relative humidity; they also bore a straight-line relationship with the logarithms of the corresponding mean mold populations.

**Acknowledgments**

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## THE DECOMPOSITION OF POTASSIUM IODATE DURING THE BAKING OF BREAD[1]

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### ABSTRACT

Potassium iodate containing radioactive iodine ($I^{131}$; half-life 8.0 days) was used to determine the fate of iodate during the baking of bread. Bread samples baked from dough containing radioactive potassium iodate were extracted with ammoniacal methanol to recover iodate and iodide. Protective carriers were added to facilitate the separation of these salts, and to minimize the extent of the side reactions of these components occurring during the extraction process. Iodide and iodate fractions, fat fractions, extracted bread solids, and oven gases were examined for radioactivity. Less than 7.5% of the original 3.5-4 p.p.m. iodate is left in the baked bread. The major decomposition product is iodide.

The improving action of a small amount of potassium bromate was first reported by Kohman *et al.* (5) in 1916. The use of potassium bromate in the maturing of flour and the improving of dough has since grown into widespread practice. Potassium bromate is supplemented with potassium iodate in certain instances for developing the optimum handling and baking qualities of dough. A combination of potassium bromate and iodate is used in the dough improver marketed under the trade- mark "Fermaloid" (8).

Although the mode of action and fate of potassium bromate in the baking process has been exhaustively studied (3, 9, 10), little is known about the behavior of iodate under similar circumstances other than that it is more readily reducible than the bromate under certain conditions (11). The purpose of the experiments described below was to ascertain the fate of potassium iodate when added to bread dough as a dough conditioner during the baking process.[2] The most likely

[1] Manuscript received November 25, 1949.
Contribution from the Research Laboratories of Merck & Co., Inc.
[2] This problem was undertaken at the request of Dr. Charles N. Frey, Director of Scientific Relations, Standard Brands Inc. Results reported herein were presented in evidence by one of the authors (C. R.) before the F.D.A. Bread Standards Hearings in Washington, D. C. on July 25, 1949.

decomposition product is potassium iodide, although other possible reactions may occur, such as iodination of fat, protein, and starch, or even volatilization of iodine.

Standard analytical procedures are complicated by the fact that only $\simeq$1.5 mg. of iodate is normally employed per lb. of bread. The determination of 0.8–0.9 mg. of iodine in $\simeq$450 g. of foreign organic material and salts is, at best, uncertain. In this case, the difficulty was magnified by the necessity for identifying in an unequivocal manner the nature of the iodine bearing components. Actual iodimetric analyses (7) of extracts from experimental loaves baked with potassium iodate failed to reveal the presence of residual iodate (cf. also ref. 11). The possibility existed, however, that residual iodate could be reduced during the extraction process prior to analysis. Thus, analyses of extracts from loaves baked without iodate but to which had been added known amounts of potassium iodide and iodate, failed to yield even approximately correct results because of uncertain and shifting endpoints. A further complication was the possibility of transformation of iodine-containing compounds in an extract by microörganisms introduced from the air, which would vitiate the interpretation of analytical data pertaining to such minute amounts of iodine. Finally, the presence of bromate in the flour or the yeast food would interfere with the determination of iodate. For these reasons, standard analytical procedures were abandoned in the solution of this problem.

An unequivocal solution to the problem, and one which would greatly simplify the analytical difficulties, is possible by the use of potassium iodate prepared from radioactive iodine ($I^{131}$; half-life 8.0 days; designated below as $\mathring{I}$). By marking in so distinctive a fashion the iodine originally added to bread dough, the decomposition products of the iodate could be located and determined unquestionably by means of Geiger counter equipment for measuring the electron emission intensity of the $I^{131}$. Possible side reactions occurring during the extraction process and in the extraction medium, which might be very significant percentage-wise when minute quantities of iodine are involved, could be minimized or swamped out by the addition of large excesses of ordinary, non-radioactive, isotopic forms of salts such as iodide and iodate. Since the presence of stable isotopic forms does not affect the activity of the minute amounts of radioactive compounds, except for self-absorption effects for which corrections can be made, the stable forms serve as carriers or protectors for the radioactive compounds. Furthermore, because the total weight of material is so greatly increased by the addition of carriers, subsequent handling becomes enormously simplified. By this carrier technique, it was

actually possible to separate the iodide and iodate fractions and to determine the radioactivity of each independently.

## Materials and Methods

*Radioactive Salts.* Radioactive potassium iodide and potassium iodate were obtained from Tracerlab,[3] Inc., Boston, Massachusetts. Purity was investigated first by iodimetric titration (7). In both cases, iodine in the form of iodate and iodide was determined. No iodide was detected in the $K\overset{*}{I}O_3$ samples nor was iodate found in the $K\overset{*}{I}$ preparations. Furthermore, emission spectrographic analysis revealed only traces of other metallic contaminants. The $I^{131}$ received from Oak Ridge was tested for isotopic purity by Tracerlab, Inc.

*Baking Experiments.* The basic formula and procedure employed in baking the radioactive loaves were as follows:

| *Ingredients and Procedure* | *Sponge* | *Dough* |
|---|---|---|
| Enriched flour | 240.0 g. | 160 g. |
| Yeast | 8.5 g. | — |
| Fermaloid (Special) | 2.0 g. | — |
| Water | 134 ml. | 100 ml. |
| Salt | — | 8 g. |
| Shortening | — | 8 g. |
| Nonfat dry milk solids | — | 12 g. |
| Fermentation time | 3.5 hr. | 1.6–2.0 hr. |
| Fermentation temperature | 80°F. | 95°F. |

Six loaves, to be referred to as A, B, C, D, N, and O, were baked with radioactive potassium iodate. Bakings A and B were preliminary experiments designed primarily to test the experimental technique and to determine whether significant amounts of volatile iodine-containing products were liberated. These loaves were baked in an electric oven, off-gases being aspirated through two scrubbers in series containing successively 10% aqueous sodium hydroxide and 3% alcoholic silver nitrate. These bakes were of somewhat inferior quality probably due to the lower temperature ($\simeq$392°–395°F.) and longer time (40–45 minutes) required for baking. Two additional loaves, C and D, were baked in a gas oven at 410°F. for 30 minutes. These loaves were in every respect normal in appearance and quality. Loaves N and O were also baked for 30 minutes in a gas oven, but at a temperature of 425°F. These loaves were also normal in appearance and quality.

All six radioactive loaves were baked with "Fermaloid" yeast food prepared[4] without potassium iodate, the latter deficiency being supplied as radioactive iodate prior to sponge preparation. About 2 mg. of $K\overset{*}{I}O_3$ was used per bake. Each radioactive loaf baked was accom-

[3] The iodine 131 used in these experiments was allocated by the Isotopes Division, U. S. Atomic Energy Commission.

[4] Courtesy of Mr. W. E. Maynard of the Fleischmann Research Laboratories.

panied by a control loaf prepared from normal "Fermaloid." Radioautographs of slices from three loaves (A, N, and O) were taken by placing these slices directly on Eastman "No-Screen" or Type K X-ray film. Fig. 1 shows the radioautographs of a center and an end slice from loaf O placed on No-Screen film two days after baking, and the film developed after an exposure of 14 days. These radioautographs show a great deal of the crumb structure because the bread slices were subjected to slight pressure during the entire period of film exposure to insure close contact with the film.

*Bread Extraction Procedure.* One-quarter loaf of baked bread A and B was dispersed in ammoniacal methanol (10 ml. of concentrated

End Slice Center Slice

FIG. 1. Radioautographs from loaf 0 (14 days exposure; "No-Screen" X-ray film).

ammonium hydroxide per 500 ml. of methanol) in a Waring Blendor. The supernatant liquid was filtered through cotton wool, and the residue reëxtracted several times with additional portions of ammoniacal methanol. This solvent was chosen in order to minimize the amount of extraneous organic material extracted with the soluble salts. Ammonia was added to prevent interaction between iodide and iodate ions, or iodate reduction in general, which occurs in acid solution. Preliminary tests showed that this solvent was adequate for dissolving the small amounts of iodine salts involved.

In the case of C and D, a half-loaf of each was extracted, the volumes of extracting solvent being proportionately larger. Furthermore, an excess of inactive reagent grade potassium iodide and potas-

sium iodate was added to the Waring Blendor before the extractions were performed. Any possibility of extraneous reaction was thus minimized as far as percentage utilization of radioactive iodine is concerned. The extraction of any adsorbed iodine salts was also facilitated.

One-quarter each of loaves N and O were extracted. Thus the initial methanolic extractant (No. 1; added portion-wise) contained the carrier salts and 20 ml. of water to insure their solution, in addition to the standard 10 ml. ammonium hydroxide; extractant No. 2 was similarly composed except for the omission of salts; and extractant No. 3 was simply the standard ammoniacal methanol solution used for

TABLE I

SUMMARY OF BAKING AND EXTRACTION DATA FOR LOAVES A–D

| Baking Data | Loaf No. | | | |
|---|---|---|---|---|
| | A | B | C | D |
| Potassium iodate added, mg. | 1.967 | 1.985 | 2.364 | 2.086 |
| Dough fermentation, proof time, min. | 68 | 70 | 75 | 75 |
| Total weight of dough, g. | 688 | 690 | 679 | 683 |
| Dough weight per loaf, g. | 500 | 500 | 500 | 500 |
| Baking temp., °F. | 392 | 395 | 410 | 410 |
| Time, min. | 45 | 40 | 30 | 30 |
| Weight of loaf, g. | 410 | 419 | 436 | 436 |
| **Extraction Data** | **A** | **B** | **C** | **D** |
| Sample of bread, weight g. | 102.5 | 105.0 | 218.0 | 218.0 |
| Volume of extractant (ml.), 1st | 400 | 250 | 325[1] | 360[1] |
| 2nd | 100 | 100 | 200 | 200 |
| 3rd | 50 | 100 | 150 | 200 |
| 4th | 50 | 50 | 100 | 200 |
| 5th | 50 | 50 | 100 | 100 |
| Total | 650 | 550 | 875 | 1060 |
| Volume extract recovered, ml. | 499 | 420 | 750 | 852 |
| Weight of dried residue, g. | 67.3 | 70.2 | 134.7 | 129.8 |

[1] Contains 10 ml. water with approximately 100 mg. KI and 100 mg. $KIO_3$.

loaves A through D. In these cases, the entire amount of inert carrier iodide and iodate was not supplied as a single addition of solid salt to the Waring Blendor (as in loaves C and D), but the carrier salt mixture was dissolved in the ammoniacal methanol with which the portionwise extraction of the bread was begun. Details relating to all bakings and extractions performed are summarized in Tables I and II.

*Separation of Free Iodide and Iodate in Bread Extracts and Dried Residues.* The determination of the small amounts of iodide and iodate present simultaneously in bread extracts was effected by converting these salts into two silver iodide precipitates, one representing the iodide and a second corresponding to the iodate fraction. These

precipitates were dissolved in aqueous sodium cyanide, and the radioactivity of the solutions measured. Knowing the specific activity (i.e., radioactivity per unit weight) and quantity of the iodate added, the percentage of initial iodine in the several fractions could be readily computed.

The separation of iodide and iodate is based upon the difference in the solubility of the respective silver salts in aqueous ammonia solution, the iodide being substantially insoluble (about 50 mg. per l.),

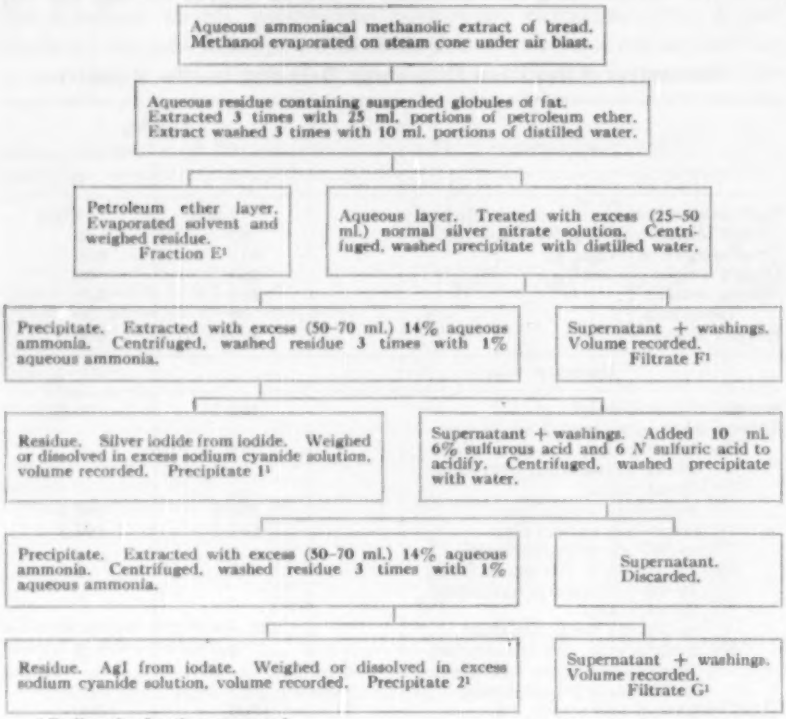
TABLE II

SUMMARY OF BAKING AND EXTRACTION DATA FOR LOAVES N AND O

| Baking Data | Loaf No. | |
|---|---|---|
| | N | O |
| Radioactive potassium iodate added, mg. | 2.068 | 2.068 |
| Dough fermentation, proof time, min. | 60 | 60 |
| Total weight of dough, g. | 677 | 680 |
| Dough weight per loaf, g. | 500 | 500 |
| Baking temp., °F. | 425 | 425 |
| Time, min. | 30 | 30 |
| Weight of loaf, g. | 409 | 400 |
| **Extraction Data** | | |
| Sample of bread, weight g. | 102.25 | 100.00 |
| Approximate number of extractions | | |
| No. 1 extractant, No. extractions | 3 | 5 |
| 10 ml. ammonium hydroxide | | |
| 20 ml. water | | |
| potassium iodate, mg. | 100.6 | 100.6 |
| potassium iodide, mg. | 101.2 | 102.2 |
| to 500 ml. with methanol | | |
| No. 2 extractant, No. extractions | 3 | 5 |
| 10 ml. ammonium hydroxide | | |
| 20 ml. water | | |
| to 500 ml. with methanol | | |
| No. 3 extractant, No. extractions | 3 | 5 |
| 10 ml. ammonium hydroxide | | |
| to 500 ml. with methanol | | |
| Total volume of extractant, ml. | 1500 | 1500 |
| Total volume of extract, ml. | 1250 | 1270 |
| Weight of dried residue, g. | 71.0 | 70.5 |

whereas the iodate is completely soluble. In order to insure complete recovery of the small amounts of radioactive iodide and iodate which might be present, weighed large amounts of reagent grade potassium iodide and iodate were added as carriers to the extract at the beginning of the separation. The separation which comprised six main steps is described in flow sheet form in Fig. 2. After removal of methanol and extraction of fat (Fraction E), silver salts (including iodide and iodate) were precipitated, and the iodate washed out with ammonia. This left

silver iodide precipitate No. 1. The ammonical extract was treated with sulfurous and sulfuric acids to reduce the iodate to iodide, which yielded a second silver iodide precipitate No. 2. Silver iodide precipitates Nos. 1 and 2 could be either collected on tared filters and weighed, or dissolved in sodium cyanide solutions. The second technique was adopted for the bulk of this work because the radioactivity measurements were considerably simplified thereby. The petroleum




[1] Radioactive fractions measured.

FIG. 2. Flow sheet for fractionation of bread extract.

ether solution containing fat Fraction E was evaporated in a tared flask and the residue weighed; the volumes of filtrates F and G were recorded. In separation test experiments E (with $K\overset{*}{I}O_3$) and H (with $K\overset{*}{I}$), water was used instead of bread extract; therefore certain steps were omitted.

During the extraction of loaves A and B, the carrier quantities of inactive potassium iodide and iodate (about 100 mg. each) were added only after the methanol and fat had been removed, since it was believed

on the basis of the literature (2) (6) and preliminary experiments with macro quantities that iodate would be stable in the bread extract if the pH was above 7. Subsequent separation tests with $KIO_3$ revealed, however, that in a methanolic bread extract prior to removal of methanol, reduction to iodide can occur on a micro scale even in the alkaline medium (experiment F). On the other hand, addition of carriers before methanol evaporation (experiment G) permitted satisfactory recovery of the radioactivity as iodate.

Similar experiments (I and R) with radioactive potassium iodide indicated that carriers had to be added to the extracts prior to methanol removal in order to minimize conversion (air oxidation?) of iodide to iodate. In view of the above experience, the inactive salts were added to extracts from loaves C, D, N, and O prior to evaporation of methanol. This precaution, coupled with the earlier addition of carrier salts to the Waring Blendor, ensured that the relative amounts of iodide and iodate left in the bread were maintained during the separation procedures, and that extraction of iodine-containing salts is essentially complete, except for possible coprecipitation effects. Assuming no loss of carriers, the total quantities added in these four experiments were about 200 mg. each of potassium iodide and iodate.

While clean, easily filterable silver iodide precipitates were obtained in runs without bread extract, the precipitates from the bread extracts were invariably contaminated with a mucilaginous material which rendered filtration very difficult. Centrifugation was successful in dealing with this problem and hence in experiments C, D, F, G, I, N, O, and R, the silver iodide[5] samples in the centrifuge tubes were dissolved by addition of a weighed amount (100–200 mg.) of reagent grade sodium cyanide and making up to a given volume (25-100 ml.).

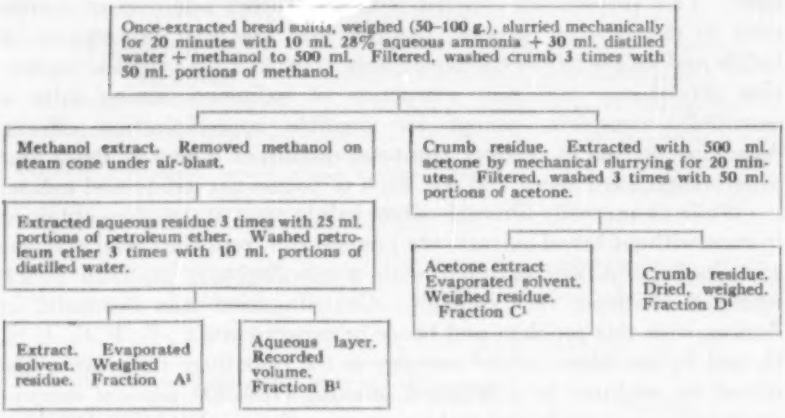
The re-extraction procedure to which residues from loaves A, D, N, and O were subjected again involved ammoniacal methanol as solvent. This was merely to ascertain the completeness of the original extraction. The extract was then separated into a petroleum ether (A) and an aqueous (B) fraction. The residue was further treated with acetone to yield another fat fraction (C) and a completely extracted residue (D). In the case of loaves D, N, and O, the aqueous extracts (B) were radioactive. Accordingly, they were examined for iodide and iodate content as mentioned above (Fig. 2) and were found to contain these salts in about the same proportion as was originally extracted. A flow sheet of the re-extraction procedure is shown in Fig. 3.

*Radioactivity Measurements.* The amount of iodine in the several fractions was determined by the radioactivity of measured portions of

[5] It should be emphasized that the purity of the silver iodides is immaterial, since only the radioactivity of precipitates Nos. 1 and 2 is being determined. It is important only to make certain that the silver iodides are completely recovered, and that contaminating impurities are not radioactive.

each sample. The activity was determined with a bell-type Geiger counter tube equipped with a thin mica window weighing 2.7 mg./cm². Samples were placed in circular stainless steel planchets 2.5 cm. in diameter and 7 mm. in depth. The activities of weighed amounts of dried residues were measured directly. Fat fractions were dissolved in a measured volume of petroleum ether or acetone, and aliquots evaporated by means of an infrared lamp. Aqueous filtrates were similarly evaporated prior to counting, as were aliquots of the sodium cyanide solutions of the silver iodides.

Activities are expressed as counts per minutes (c.p.m.) and are corrected for background and for the decay of radioactive iodine using a half-life of 8.0 days. For each baking experiment or separation test, a zero time was chosen, and all activities were corrected for iodine decay




[1] Radioactive fractions measured.

FIG. 3. Flow sheet for re-extraction of bread residue.

back to this starting time. The amount of iodine present in any given fraction could then be expressed as a percentage of the total iodine. No coincidence corrections were made since relatively low activities were measured throughout. During the bulk of this work, a National Bureau of Standards Radium D + E standard preparation was measured daily as a check on sensitivity variations of the counter tube, and appropriate corrections made for daily variations.

Every fraction possessing significant activity was usually measured in triplicate. Furthermore, each measurement involved a different amount of sample, i.e. varying aliquots of solution or several weights of residue. This was done to ascertain whether the residues were thick enough to absorb an appreciable part of the electrons emitted by the radioiodine. In the case of the fat fractions, and of course in the dried,

extracted bread residues, self-absorption (1, 4, 12) of radiation was important, and appropriate correction factors were established experimentally. The above correction data are not reported since they apply strictly to the particular measuring equipment employed.

*Standardizations and Separation Tests.* The radioactivity of the stock $K\overset{*}{I}O_3$ and $K\overset{*}{I}$ salts was determined by weighing several mgm. of salt into a 50–100 ml. volumetric flask, dissolving in water, and measuring the activity of the aliquot residues as described in the preceding section. The specific activities of these salts, expressed as counts per min. per mgm., were calculated for those times taken as the starting points of pertinent separation and baking experiments.

The efficiency of the iodide-iodate separation procedure was tested separately with radioactive potassium iodide and with radioactive

TABLE III

TESTS OF SEPARATION EFFICIENCY

| Expt. | Radioactive Component | Medium | Carrier added | % of Total Measured Activity | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | Iodide | Iodate | Fat Fraction | Filtrate F | Filtrate G |
| E | Iodate | Water | Present | 4.4 | 95.6 | — | Discarded | Discarded |
| F | Iodate | Bread Extract | After Methanol Removal | 87.6 | 11.2 | 0.4 | 0.3 | 0.3 |
| G | Iodate | Bread Extract | Before Methanol Removal | 7.3 | 92.4 | 0.3 | Discarded | Discarded |
| H | Iodide | Water | Present | 99.3 | 0.7 | — | Discarded | Discarded |
| I | Iodide | Bread Extract | After Methanol Removal | 89.7 | 9.9 | 0.4 | Discarded | Discarded |
| R | Iodide | Bread Extract | Before Methanol Removal | 97.0 | 2.4 | 0.1 | 0.4 | 0.1 |

potassium iodate added to water and to ammoniacal methanol extracts of bread. Results are reported in Table III which gives the activities of the various fractions in per cent of total activity. The theoretical activity can be calculated for any experiment from the weight of radioactive salt employed and its specific activity. Because of the difficulties inevitably attending these radioactivity measurements, and because of the large number of different samples measured, the measured total activity may deviate significantly from the theoretical activity. Whenever this difference exceeded 1% in these separation tests and in the baking experiments reported in the next Section, the per cent of total activity in the several fractions was recomputed on the basis of the measured total activity as 100%.

This permits a more convenient comparison of the distribution of activity among the different fractions of a given experiment.

Three experiments[6] were performed with the iodate. Experiment E was carried out in water in the presence of added inert iodide and iodate as a preliminary trial of the validity of the separation. Apparently a small amount (4.4%) of the initial active potassium iodate is retained by the iodide fraction. In this test the silver iodide precipitates from the iodide and iodate fractions were filtered and weighed prior to solution in cyanide for determination of radioactivity. The weights of both precipitates were about 99% of theoretical.

Experiment F is the counterpart of E made on a bread extract. Excess salts were not added, however, until after evaporation of the methanol. No attempt was made to weigh the silver iodide precipitates prior to solution in cyanide. It is obvious that considerable reduction ($\simeq$88%) of the minute amount of iodate to iodide occurred during the removal of the methanol from the extract.

In experiment G, also performed with an ammoniacal methanol bread extract, the carrier salts were added before evaporation of methanol in order to minimize the extent of possible iodate reduction. The effectiveness of this swamping procedure is demonstrated by the fact that only about 7% of the radioactive iodine, introduced originally as iodate, was present in the iodide fraction. This may be due more to coprecipitation effects enhanced by the presence of protein, starch, and salts in the extract than to actual reduction of iodate by bread components.

Experiments[7] H, I, and R were performed with radioactive potassium iodide. In water (experiment H) the separation is virtually quantitative; and the weights of silver iodide precipitates Nos. 1 and 2 prior to solution in cyanide agree with the expected weights. In the presence of bread extract, however, a small amount of iodide is retained by the iodate fraction. Thus, in experiment I in which the inert carriers were added after removal of methanol, this amounts to $\simeq$10% and may represent a small degree of (air ?) oxidation of iodide to iodate in basic solution. This effect is practically eliminated, however, by supplying the carrier salts at the very start of the separation procedure, i.e. before evaporation of the alcohol. Thus experiment R shows that only 2.4% of the total activity resides in the iodate fraction. The protecting effect of the carrier in minimizing the extent of undesirable reactions of the small amounts of active material present, until a final separation can be achieved, is thus again demonstrated.

The above series of separation tests shows the necessity for addition of protective carrier salts at the very start of the separation procedure.

[6] $\simeq$100 mg. of each carrier salt used in these experiments.
[7] 100 mg. of each carrier used in experiments H and I; 200 mg. of each used in experiment R.

Although the separations of the iodide and iodate fractions are not clean from a quantitative point of view, the extent of contamination of iodide by iodate and vice versa has been evaluated in the separation tests. This will in no way invalidate the general conclusions as to the course of the iodate decomposition, but will tend to yield somewhat high iodate values.

## Results

The distribution of radioactivity among the several extraction fractions and residues from the six loaves of bread baked with radioactive potassium iodate is reported in Tables IV-VI. Successive columns represent the number and nature of the fraction involved, the radioactivity in c.p.m., the per cent of theoretical total activity of the

TABLE IV

PRELIMINARY BAKING EXPERIMENTS A AND B; ELECTRIC OVEN

| Fraction | | Experiment A[1] | | | Experiment B[3] | |
|---|---|---|---|---|---|---|
| Number | Nature | c.p.m. | % of Theoretical Activity | % of Measured Total Activity | c.p.m. | % of Theoretical Activity[4] |
| — | Off-gas | 0 | 0 | 0 | 100 (?) | 0.02 (?) |
| 1 | Iodide | 330,500 | 59.7 | 65.4 | 176,000 | 40.9 |
| 2 | Iodate | 23,300[2] | 4.2 | 4.6 | 63,640 | 14.8 |
| E | Fat Fraction | 2,610 | 0.5 | 0.5 | 3,730 | 0.9 |
| F | Filtrate | 570 | 0.1 | 0.1 | 800 | 0.2 |
| G | Filtrate | 980 | 0.2 | 0.2 | 570 | 0.1 |
| — | Extracted Residue | 147,000 | 26.6 | 29.1 | 116,700 | 27.2 |
| A | Fat (Pet. ether) | 5,150 | (0.9) | (1.0) | — | — |
| B | Aqueous Fraction | 990 | (0.2) | (0.2) | — | — |
| C | Fat (Acetone) | 10,300 | (1.9) | (2.1) | — | — |
| Total Measured Activity | | 504,960 | 91.3 | 100 | 361,440 | 84.1 |
| Theoretical Activity | | 553,000 | 100 | 109.5 | 430,000 | 100 |
| Deficiency | | 98,040 | 8.7 | — | 68,560 | 15.9 |

[1] Calculated initial weight $K\overset{*}{I}O_3$ in bread sample 0.358 mg. Carriers added after removal of methanol (100.3 mg. iodate; 100.8 mg. iodide).
[2] Corrected for loss of precipitate No. 2 as described in test.
[3] Calculated initial weight $K\overset{*}{I}O_3$ in bread sample 0.360 mg. Carriers added after removal of methanol (100.0 mg. iodate; 100.3 mg. iodide).
[4] % of measured total activity not computed because this activity balance sheet is obviously deficient.

bread sample, and the per cent of the measured total activity present in a given fraction, recalculated as described in the preceding section. Figures in parentheses are not included in the totals.

Table IV summarizes results obtained with the loaves (experiments A and B) baked in an electric oven. These preliminary experiments are atypical because of the non-uniform heat and lower temperatures

prevailing in the oven employed for baking. Furthermore, in the extraction of these loaves, carrier salts were not added until after methanol was removed from the bread extract, so that the values of iodate and iodide activities are in doubt. In both cases an attempt was made to collect and weigh the silver iodide precipitates prior to solution in cyanide for activity determination. In contrast to the virtually complete recoveries obtained from water solutions (experiments E and H), silver iodide precipitate weights tended to run con-

TABLE V

BAKING EXPERIMENTS C AND D; GAS OVEN

| Fraction | | Experiment C[1] | | | Experiment D[2] | | |
|---|---|---|---|---|---|---|---|
| Number | Nature | c.p.m. | % of Theoretical Activity | % of Measured Total Activity | c.p.m. | % of Theoretical Activity | % of Measured Total Activity |
| 1 | Iodide | 39,050 | 71.7 | 72.8 | (34,800) | (72.8) | (75.1) |
| 2 | Iodate | 1,650 | 3.0 | 3.0 | (1,650) | (3.4) | (3.6) |
| E | Fat Fraction | 20 (?) | 0.04 (?) | 0.04 (?) | 0 | 0 | 0 |
| F | Filtrate | 100 (?) | 0.2 (?) | 0.2 (?) | 0 | 0 | 0 |
| G | Filtrate | 20 (?) | 0.04 (?) | 0.04 (?) | 0 | 0 | 0 |
| — | Extracted Residue | 12,900 | 23.6 | 23.9 | — | — | — |
| A | Fat. (Pet. Ether) | — | — | — | 0 | 0 | 0 |
| C | Fat (Acetone) | — | — | — | 200 (?) | 0.4 (?) | 0.4 (?) |
| D | Re-extracted[3] Residue | — | — | — | 0 | 0 | 0 |
| 1 | Iodide (2nd Extraction) | — | — | — | (9,100) | — | — |
| 2 | Iodate (2nd Extraction) | — | — | — | (600) | — | — |
| — | Total Iodide | — | — | — | 43,900 | 91.8 | 94.8 |
| — | Total Iodate | — | — | — | 2,250 | 4.7 | 4.8 |
| Total Measured Activity | | 53,750 | 98.6 | 100 | 46,350 | 96.8 | 100 |
| Theoretical Activity | | 54,500 | 100 | 101.4 | 47,870 | 100 | 103.3 |
| Deficiency | | 750 | 1.4 | — | 1,520 | 3.2 | — |

[1] Calculated initial weight $K\overset{*}{I}O_3$ in bread sample 0.870 mg. Carriers added at time of extraction (≅100 mg. each) and before methanol evaporation (≅100 mg. each).

[2] Calculated initial weight $K\overset{*}{I}O_3$ in bread sample 0.764 mg. Carriers added at time of extraction (≅100 mg. each) and prior to methanol evaporation (≅100 mg. each).

[3] Re-extraction in the presence of ≅50 mg. of iodate and ≅50 mg. iodide.

siderably higher than theoretical due to the presence of mucilaginous contaminants which adhered to the precipitates. The single exception occurred in experiment A where, due to peptization, the recovery of the precipitate No. 2 corresponding to the iodate fraction was only 76.5%. The c.p.m. value listed for this fraction in Table IV is corrected for this loss.

The tendency of the ammonia-extracted silver iodide residues from bread extract to peptize when washed was always extremely troublesome, and in some cases the iodides could not be centrifuged after

washing unless some sodium nitrate was added to the supernatant as coagulant. The peptization was caused exclusively by bread colloids, since it was never observed in silver iodide residues from pure water solutions.

An attempt to re-extract the residue from experiment A with ammoniacal methanol yielded but little additional activity. It was found subsequently that extraction efficiency was increased by the presence of carriers, and that re-extraction by a slurrying procedure was more effective. The recovery of silver iodide precipitates by

TABLE VI

BAKING EXPERIMENTS N AND O; GAS OVEN

| Fraction | | Experiment N[1] | | Experiment O[3] | |
|---|---|---|---|---|---|
| Number | Nature | c.p.m. | % of Theoretical Activity | c.p.m. | % of Theoretical Activity |
| 1 | Iodide | (145,200) | (87.5) | (141,600) | (85.7) |
| 2 | Iodate | (4,340) | (2.6) | (12,400) | (7.5) |
| E | Fat Fraction | ≃1,400 | 0.8 | 125 | 0.1 |
| F | Filtrate | ≲5,860 | 3.5 | ≃700 | 0.4 |
| G | Filtrate | 930 | 0.5 | 730 | 0.4 |
| A | Fat (Pet. Ether) | ≃50 | 0 | — | — |
| D | Re-extracted Residue[2] | ≃5,400 | 3.3 | 6,700 | 4.1 |
| 1 | Iodide (2nd Extraction) | (3,540) | — | (2,920) | — |
| 2 | Iodate (2nd Extraction) | (≃130) | — | (100) | — |
| — | Total Iodide | 148,740 | 89.5 | 144,520 | 87.4 |
| — | Total Iodate | 4,470 | 2.7 | 12,500 | 7.5 |
| Total Measured Activity | | 166,850 | 100.4 | 165,275 | 99.9 |
| Theoretical Activity | | 166,200 | 100 | 165,300 | 100 |
| Excess (N) or Deficiency (O) | | 650 | 0.4 | 25 | 0.1 |

[1] Calculated initial weight $K^{*}IO_3$ in bread sample 0.382 mg. Carriers added at time of extraction (≃100 mg. each) and before evaporation of methanol (≃100 mg. each).
[2] Re-extraction in the presence of ≃50 mg. iodate and iodide.
[3] Calculated initial weight $K^{*}IO_3$ in bread sample 0.380 mg. Carriers added at time of extraction (≃100 mg. each) and before evaporation of methanol (≃100 mg. each).

filtration appears to be of doubtful value since, in both experiments A and B, large activity deficiencies (differences of 9 and 16% between theoretical and total measured activities) were noted. Accordingly, in subsequent experiments the filtration practice was discontinued, and all washings were performed in centrifuge tubes. The justification for this step is seen from the improved activity balance sheets characterizing the remaining experiments. It is obvious, however, even from these preliminary experiments, that a negligible amount of iodine is liberated among the gaseous products, and that the reduction of iodate during baking is extensive.

Tables V and VI summarize the results obtained with the four loaves (experiments C, D, N, and O) baked in a gas oven. All four cases illustrate the increased initial extraction efficiency in the presence of carriers, which were added before evaporation of methanol. In the last three experiments, the once-extracted residues were re-extracted by a slurrying technique in the presence of carrier. By this device, additional iodate and iodide were obtained, in about the same ratio as in the initial extractions. In experiment N, filtrate F exhibited an exceptionally high (3.5%) activity. This is probably due to incomplete precipitation of the iodate and/or iodide. Accordingly, the value of 2.7% reported in this experiment for iodate is probably low, and may actually be as high as 6.2%.

The uniform distribution of the radioactive salts in the loaves and in the samples taken for analysis was demonstrated qualitatively by the radioautographs of end and center slices (Fig. 1). The excellent radioactivity balance sheets obtained with unequivocal bakings C, D, N, and O constitute quantitative evidence of proper sampling.

## Discussion

In all of these experiments, the largest fraction of total activity present as iodate was 14.8%. This occurred in the preliminary experiment B, which was atypical because of unrepresentative baking conditions and was suspected on the grounds of incomplete separation and because of a very incomplete activity balance. In the remaining baking experiments, which included the four unequivocal bakings C, D, N, and O, the amount of iodine found as unconverted iodate was considerably less, i.e. 4.6, 3.0, 4.8, 6.2, and 7.5%. This leads to the conclusion that less than 7.5% of the original iodate is left in this form after the baking process. Even this value is undoubtedly high because the separation of iodide from iodate was not quantitative (see section on separation tests). The retention of iodate by iodide would not affect the results appreciably because of the small amount of iodate involved. Since the bulk of the radioactivity is present as iodide, however, the presence of several per cent of iodide in the iodate fraction (cf. experiment R) would lead to significantly higher apparent values for iodate.

It appears likewise that the principal iodine-containing product formed is potassium iodide. One might be inclined to deduce from experiments A, B, and C (Tables IV and V) that only about half or two-thirds of the original iodine is converted to iodide, and that a considerable fraction (24–27%) is permanently fixed in the dried residue. However, experiments D, N, and O (Tables V and VI), in which more efficient extractions were performed, show quite clearly

that the activity of the once extracted bread is not permanently bound but, on the contrary, extractable; actually about 90% of the initial iodate is transformed to iodide. Although the small amount of activity (3-4%) left in the re-extracted residues (experiments N and O) may actually be retained as combined iodine, it is more likely present simply as adsorbed salt, hence completely extractable as was found in experiment D. No radioactivity was observed in the scrubbed off-gases; and the activities of the fat fractions were small and variable, and probably were due to slight contamination by the active aqueous phases extracted. It is evident from the above experiments that potassium iodate is extensively decomposed to potassium iodide during the baking of bread.

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# BAKING PROPERTIES AND PALATABILITY STUDIES OF SOY FLOUR IN BLENDS WITH HARD WINTER WHEAT FLOUR[1]

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**ABSTRACT**

A commercially milled and bleached hard red winter wheat flour containing 13.9% protein was baked with 4% and 8% of a commercial hexane-extracted, a commercial full-fat, and an ethanol extracted soy flour. Excellent bread was made with blends containing up to 8% soy flour with the hard red winter wheat flour, the quality of the bread as measured by crumb grain and loaf volume being equal to that for wheat flour alone provided the quantity of potassium bromate used in the baking formula was increased. The average increase in baking absorption for each 1% of soy flour added was over 1%. Crumb colors for bread containing soy flour were creamy-gray, the degree of which was dependent on the kind and amount of soy flour used. Soy flours have specific baking properties and potentialities in blends with hard red winter wheat flour. These potentialities are not expressed, however, unless suitable alterations are made in the baking method.

For palatability studies, bread was made from wheat flour alone and from wheat-soy flour blends containing 4% and 8% of two commercial hexane-extracted, two commercial full-fat, and two ethanol-extracted soy flours. These breads were scored for tenderness, soylike flavor, desirability of flavor, and acceptability of bread. Statistical analysis of the data obtained in this study indicated that bread made from wheat flour alone was preferred to that made from soy-wheat flour blends. In addition, one of the hexane soy flours was more easily detected than the other at the 8% level; 8% of soy flour was more easily detected than 4%; and ethanol-extracted soy flours were preferred to full-fat flours at the 8% level.

The addition of 1 mg. of potassium bromate per 100 g. of wheat flour by Bohn and Favor (2) improved loaf volume and internal characteristics of the bread made from wheat-soy flour blends. Studies carried out in the Soft Wheat Quality Laboratory at Wooster, Ohio (3) showed that bread of excellent loaf volume and crumb grain could be made from blends containing up to 8% soy flour with hard red spring wheat flour, providing the quantity of potassium bromate used in the baking formula was increased with the amount of soy flour used.

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Bayfield and Swanson (1) state that it was necessary to increase potassium bromate with increasing percentages of soy flour, and that when proper adjustments in formula and procedure were made, bread containing as much as 6% soy flour was equal to the control, except for crumb color. These breads, however, were not judged for palatability. Accordingly, the cooperation of the Bureau of Human Nutrition and Home Economics was requested to study the acceptability of such breads.

A hard red *winter* wheat flour was used for the palatability studies and they in turn were expanded sufficiently to obtain data comparable to those reported by Finney (3), in previous studies with soy flour in blends with a hard red *spring* wheat flour. In this way a comparison was made of the baking properties of the two classes of hard wheat flour, winter and spring, when in blends with soy flour. This paper describes the baking properties and behavior of blends containing 4% and 8% soy flour with hard red winter wheat flour containing 13.9% protein, and outlines the judging procedure employed and gives a statistical summary of data obtained in making the acceptability tests.

## Materials and Methods

Two commercial hexane-extracted (A and B), two commercial full-fat (C and D), and two ethanol-extracted (E and F) soy flours prepared by the Northern Regional Research Laboratory were used in the breads made for palatability tests. Three composite soy flours (A + B, C + D, and E + F), however, were used in the studies concerned with evaluating the bread baking properties of the three types of soy flour. These three composites of soy flour contained 43.0%, 31.9% and 46.6% protein, respectively. Four and 8% of each of these three composite soy flours were blended with a commercially milled and bleached hard red winter wheat flour containing 13.9% protein. Each of these blends (100 g. wheat flour plus soy flour) was baked with 0, 3, 4, and 5 mg. of potassium bromate per 100 g. flour and with 4% nonfat milk solids in the formula. Wheat flour alone was baked into bread with 0, 2, 3, and 4 mg. of bromate and 4% nonfat milk solids, and with 0, 1, 2, and 3 mg. of bromate when the milk solids were omitted. Additional ingredients used in all bakes were 6 g. sugar, 1.5 g. salt, 3 g. shortening, 2 g. yeast, 0.25 g. malt syrup (120°L), and water as needed. An optimum mixing time with the straight dough procedure and a 3-hr. fermentation at 30°C. were employed. All punching and panning were performed mechanically. Baking time was 25 min. at 221°C. Bakings were replicated at least twice. A third replicate was made when loaf volumes differed by more than 25 cc. Data for all replicates were averaged.

Each series of samples for palatability tests included six loaves containing 4% and 8% of three of the six soy flours, two loaves duplicating one of the soy flours at each of the two percentage levels, and one check loaf of wheat flour only. Twelve replications of this nine loaf series were baked in the Federal Soft Wheat Laboratory at Wooster, Ohio, using the above formula together with 4% nonfat milk solids and optimum bromate. Immediately after cooling, each nine-loaf series was sent by air to the laboratory at Beltsville for the palatability tests. The elapsed time between baking and the palatability tests was approximately 48 hr.

The palatability tests were preceded by four training periods in which judges were taught to recognize soy flavor, to judge tenderness, and to score these two factors quantitatively. On another part of the score card they were asked to state preferences for samples in terms of the desirability of flavor and general acceptability of the bread. Thus, information on amount of soy flavor detected in breads containing the various kinds of flours was recorded independent of whether or not the flavor was enjoyed.

The samples presented at each of 12 judging sessions included coded slices of bread containing no soy flour and 4% and 8% of one hexane-extracted, one full-fat, and one ethanol-extracted soy flour. Labeled slices of bread made from wheat flour alone were included for the purpose of comparison. The judging room was partially darkened to minimize color and texture differences. Cool water and slices of apple were allowed as needed to clear the mouth of bread flavors.

The presentation of samples during the 12 bread-judging periods was such that each judge made eight scorings for tenderness, soylike flavor, desirability of flavor, and acceptability of bread for each of the six soy flours at each per cent level and 12 scorings for wheat flour alone. Oily, musty, rancid, sour, and bitter were adjectives listed for describing bread flavors. Tenderness was scored from five (normal) to three (very tender or very tough), soylike flavor from five (none) to one (very strong), and desirability of flavor and acceptability of bread from five (very good) to one (not acceptable). Student's "t" test was used to test for significance between various combinations of mean scores.

## Results

The baking absorptions, mixing times, potassium bromate requirements, and bread-crumb color scores for the 13.9% protein hard winter wheat flour alone and when blended with 4% and 8% of the three different soy flours are given in Table I, and the loaf volumes and crumb grain scores are shown graphically in relation to the potassium

TABLE I

ABSORPTIONS, MIXING TIMES, and POTASSIUM BROMATE REQUIREMENTS FOR HARD RED WINTER WHEAT FLOUR DOUGHS CONTAINING 0%, 4% AND 8% OF 3 DIFFERENT SOY FLOURS TOGETHER WITH THE CRUMB COLOR SCORES FOR BREAD BAKED THEREFROM

| Kind and Per Cent of Soy-Flour in Blends | Baking[1] Absorption | Mixing Time | Potassium Bromate Requirement | Optimum Bread-Crumb Color[2] |
|---|---|---|---|---|
| | % | *min.* | *mg.* | |
| 4% Soy Flour | | | | |
| Com'l oil-free A + B[3] | 74.3 | 2¾ | 4 | 87 |
| Com'l full-fat C + D | 73.1 | 2¾ | 4 | 85 |
| Ethanol-extracted E + F | 75.9 | 2¾ | 4 | 86 |
| 8% Soy Flour | | | | |
| Com'l oil-free A + B | 78.4 | 2¾ | 5 | 81 |
| Com'l full-fat C + D | 75.8 | 2¾ | 5 | 73 |
| Ethanol-extracted E + F | 81.2 | 2¾ | 4 | 74 |
| No Soy Flour | | | | |
| Control | 70.1 | 2¾ | 3 | 103 |
| Nonfat M.S. omitted[4] | 67.6 | 2¾ | 1 | 107 |

[1] 14% moisture. When bromate was omitted in the formula, absorptions were reduced 1%.
[2] Loaves having the optimum crumb grains and loaf volumes, in general, had the best crumb colors.
[3] Equal parts of each at 14% moisture.
[4] All other doughs contained 4% nonfat milk solids.

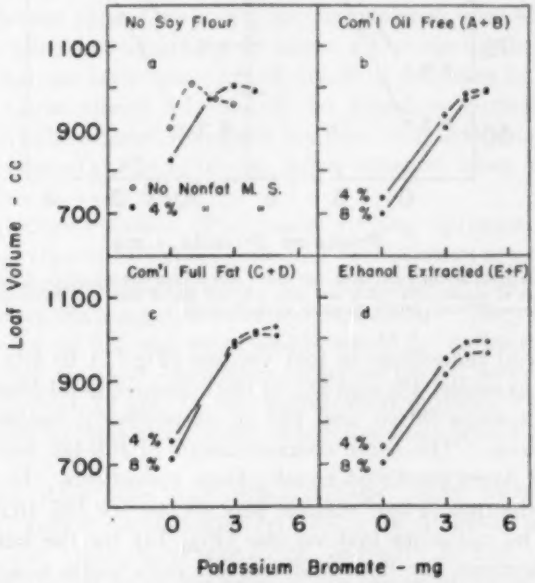


FIG. 1. Loaf volumes for a hard red winter wheat flour (13.9% protein) baked with and without the addition of nonfat milk solids and with 4% and 8% of each of 3 different soy flours at several potassium bromate levels with milk solids in the formula.

bromate requirements in Figs. 1 and 2. The bromate requirement given in Table I is the amount of potassium bromate required to produce approximately the maximum loaf volume shown in Fig. 1.

*Loaf Volume and Bromate Requirement.* The wheat flour without soy flour and without milk solids produced a loaf volume of 910 cc. (Fig. 1a) with no potassium bromate in the formula. The addition of 4% nonfat milk solids, however, reduced loaf volume 80 cc. When the hard winter wheat flour was blended with 4% and 8% of soy flour and baked into bread by the same formula (4% milk solids and no bromate)

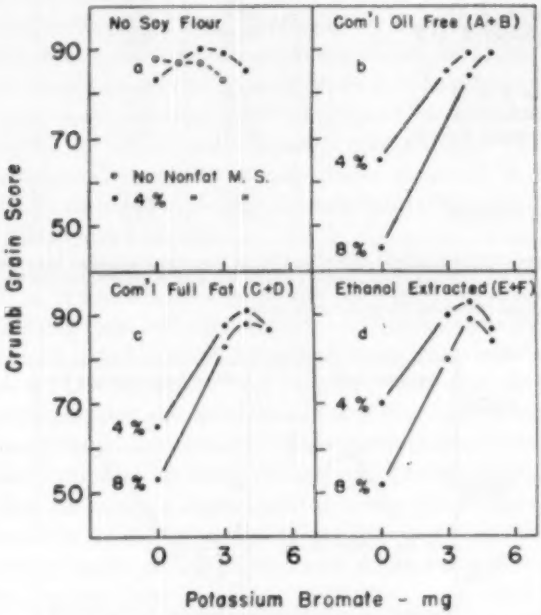


FIG. 2. Crumb grain scores for a hard red winter wheat flour (13.9% protein) baked with and without the addition of nonfat milk solids and with 4% and 8% of each of 3 different soy flours at several potassium bromate levels with milk solids in the formula.

further material reductions in loaf volume (Fig. 1a to 1d) were obtained. For example, 4% and 8% of the commercial oil-free soy flour reduced loaf volume 90 cc. and 127 cc. respectively, below that for milk solids alone. The same concentrations of full-fat and ethanol-extracted soy flours produced equally large reductions. In every instance the reduction in loaf volume was greater for 8% than for 4% soy flour. The optimum loaf volume (Fig. 1a) for the hard winter wheat flour without soy flour and without milk solids was produced with only 1 mg. of potassium bromate; whereas with 4% milk solids 3 mg. were required. When 4% and 8% of each of the three soy

flours were included with milk solids in the formula, 4 to 5 mg. of bromate were required for optimum loaf volume (Fig. 1b, 1c, and 1d), although the commercial full-fat soy flour gave nearly optimum volumes equal to that for milk solids alone with only 3 mg. of bromate. The optimum volumes for both concentrations of all three soy flours were equal to or slightly greater than the optimum for milk solids alone, except the 8% ethanol-extracted soy flour.

*Bread Crumb Grain.* The bread crumb grain data (Fig. 2) are principally of interest from the standpoint of a comparison with loaf volumes obtained at corresponding potassium bromate levels. First it will be noted that additions of each of the soybean flours strikingly decreased the crumb grain scores (Fig. 2b, 2c, and 2d) as compared with the hard winter wheat flour alone (Fig. 2a) when no bromate was used, and that this depressing or reducing effect, also noted for loaf volume, was much greater for 8% than for 4% soy flour. These scores, however, gradually improved as the quantity of bromate increased, in the same manner as was noted for loaf volume, so that the loaf volume and crumb grain curves roughly parallel each other. Most important, the optimum crumb grains and optimum loaf volumes were produced with approximately the same quantity of bromate in the formula. In addition, the optimum crumb grains for the 4% soy flour blends were equal to or slightly better than those obtained for wheat flour alone; whereas those for the 8% soy flour blends were slightly poorer. Thus the effects on crumb grain scores are so similar to those on loaf volume that the latter may for all practical purposes be used alone to evaluate these effects. It should be noted, nevertheless, that the blends with the ethanol-extracted soy flour (Fig. 2d) are more sensitive to the detrimental effects of too much bromate than are the blends of the other two soy flours.

*Bread Crumb Color.* The loaves having optimum crumb color scores (last column of Table I) also had the optimum or nearly optimum loaf volumes and crumb grains. The crumb color value of 103, which characterizes the control, is considered excellent. The crumb colors of 85 to 87 for all 4% soy flour blends would be noticeably creamy or creamy-gray to some consumers of white bread; whereas the bread crumb colors of 73 and 74 for the blends containing 8% of the commercial full-fat and ethanol-extracted soy flour would be noticed by many.

*Baking Absorption and Mixing Time.* The water absorptions (Table I) varied considerably for the three different soy flour composites. For example, it was found necessary to add 1.05, 0.75, and 1.45% additional water for each 1% of the commercial oil-free, commercial full-fat, and ethanol-extracted soy flour, respectively. Mix-

ing requirement of the hard winter wheat flour was not altered by the addition of soy flour.

The mean scores for soylike flavor, desirability of flavor, and acceptability of bread together with the levels of significance for differences between types and percentages of soy flour are given in Tables II, III, and IV.

*Tenderness.* There seemed to be no agreement among judges as to whether samples were more tender or more tough than normal.

TABLE II

STATISTICAL SIGNIFICANCE OF DIFFERENCES IN STRENGTH OF SOY-LIKE FLAVOR

| Comparisons | Sample and % Level of Soy | Mean Score | Mean Score | Sample and % Level of Soy | Significance |
|---|---|---|---|---|---|
| a) Within type at each level | $A_4$ | 4.04 | 4.00 | $B_4$ | — |
| | $A_8$ | 3.94 | 3.50 | $B_8$ | ** |
| | $C_4$ | 3.88 | 3.98 | $D_4$ | — |
| | $C_8$ | 3.47 | 3.64 | $D_8$ | — |
| | $E_4$ | 4.29 | 4.04 | $F_4$ | — |
| | $E_8$ | 3.96 | 3.75 | $F_8$ | — |
| b) Between wheat and soy flour at each level | Mean score of wheat (4.72) significantly different from mean score of each of the soy samples.** | | | | |
| c) Between levels within a type | $(A+B)_4$ | 4.02 | 3.94 | $A_8$ | — |
| | $(A+B)_4$ | 4.02 | 3.50 | $B_8$ | ** |
| | $(C+D)_4$ | 3.93 | 3.56 | $(C+D)_8$ | ** |
| | $(E+F)_4$ | 4.15 | 3.85 | $(E+F)_8$ | ** |
| | $B_4$ | 4.00 | 3.50 | $B_8$ | ** |
| d) Between types within a level | $(A+B)_4$ | 4.02 | 3.93 | $(C+D)_4$ | — |
| | $(A+B)_4$ | 4.02 | 4.15 | $(E+F)_4$ | — |
| | $(C+D)_4$ | 3.93 | 4.15 | $(E+F)_4$ | — |
| | $(C+D)_8$ | 3.56 | 3.85 | $(E+F)_8$ | * |
| | $(C+D)_8$ | 3.56 | 3.94 | $A_8$ | * |
| | $(C+D)_8$ | 3.56 | 3.50 | $B_8$ | — |
| | $(E+F)_8$ | 3.85 | 3.94 | $A_8$ | — |
| | $(E+F)_8$ | 3.85 | 3.50 | $B_8$ | * |

— Not significant.
* Significant at 5% level.
** Significant at 1% level, or highly significant.

Some samples, including wheat, received "tender," "tough," and "normal" ratings on the same day. The wheat samples had a grand mean of 4.81 in comparison with a "normal" tenderness score of 5.0 and the soy samples ranged from 4.70 for the 4% level of one ethanol flour to 4.92 for the 4% level of the other. Because the deviation from the normal was so small, the test of significance was not applied to these mean scores.

*Soylike Flavor.* Judges readily distinguished between the wheat samples and any soy sample, as is shown by a comparison of the mean

scores in Table II (a and b). The mean score of 4.72 for wheat was significantly different from the mean score for the 4% and 8% levels of each of the soy samples (P = 0.01). Differences were not significant between two flours of the same kind (Table IIa) except for the two hexane flours (A and B) at the 8% level. This difference suggests that the hexane process may vary from one processor to another.

The 8% level of each soy flour had significantly more soylike flavor than the 4% level (Table IIc) except for hexane A, the scores for which were 4.04 for the 4% and 3.94 for the 8% levels. None of the differ-

TABLE III

STATISTICAL SIGNIFICANCE OF DIFFERENCES IN DESIRABILITY OF FLAVOR

| Comparisons | Sample and % Level of Soy | Mean Score | Mean Score | Sample and % Level of Soy | Significance |
|---|---|---|---|---|---|
| a) Within type at each level | $A_4$ | 4.24 | 4.25 | $B_4$ | — |
| | $A_8$ | 4.12 | 3.91 | $B_8$ | — |
| | $C_4$ | 4.04 | 4.28 | $D_4$ | — |
| | $C_8$ | 3.59 | 3.98 | $D_8$ | — |
| | $E_4$ | 4.38 | 4.23 | $F_4$ | — |
| | $E_8$ | 4.27 | 4.04 | $F_8$ | — |
| b) Between wheat and soy flour at each level | Mean score of wheat (4.77) significantly different from mean score of each of soy samples.** | | | | |
| c) Between levels within a type | $(A+B)_4$ | 4.24 | 4.01 | $(A+B)_8$ | — |
| | $(C+D)_4$ | 4.17 | 3.80 | $(C+D)_8$ | ** |
| | $(E+F)_4$ | 4.30 | 4.15 | $(E+F)_8$ | — |
| d) Between types within and between levels | $(A+B)_4$ | 4.24 | 4.17 | $(C+D)_4$ | — |
| | $(A+B)_4$ | 4.24 | 4.30 | $(E+F)_4$ | — |
| | $(C+D)_4$ | 4.17 | 4.30 | $(E+F)_4$ | — |
| | $(A+B)_4$ | 4.24 | 4.15 | $(E+F)_8$ | — |
| | $(A+B)_8$ | 4.01 | 3.80 | $(C+D)_8$ | — |
| | $(A+B)_8$ | 4.01 | 4.15 | $(E+F)_8$ | — |
| | $(C+D)_8$ | 3.80 | 4.15 | $(E+F)_8$ | * |
| | $(E+F)_4$ | 4.30 | 4.01 | $(A+B)_8$ | * |

— Not significant.
* Significant at 5% level.
** Significant at 1% level, or highly significant.

ences between the scores for the 4% soy flour levels (Table IId) were statistically significant, although there was a little less soylike flavor in the ethanol-extracted soy flour than in either of the other two types. At the 8% level, however, the full-fat flours differed significantly from one of the hexane flours (A) and the two ethanol flours (E and F), being more pronounced in soylike flavor than any of them. One of the hexane soy flours (B) had a significantly more soylike flavor than the ethanol flours E and F; whereas the other did not.

*Desirability of Flavor.* According to the judges, the bread baked

with all wheat flour had significantly more desirable flavor than any of the soy breads. The mean score for desirability of flavor of the wheat sample was 4.77 (Table IIIb) and the highest mean for any soy sample was 4.38 ($E_4$, Table IIIa), indicating that in this study bread baked from wheat flour alone was preferred to that containing 4% or 8% of the three types of soy flours. There was no significant difference within 4% levels or within 8% levels of flours of the same type (Table IIIa). The 4% levels of the full-fat soy flours (C + D, Table IIIc) had a significantly more desirable flavor than the 8% levels;

TABLE IV

STATISTICAL SIGNIFICANCE OF DIFFERENCES IN ACCEPTABILITY OF BREAD

| Comparisons | Sample and % Level of Soy | Mean Score | Mean Score | Sample and % Level of Soy | Significance |
|---|---|---|---|---|---|
| a) Within type at each level | $A_4$ | 4.25 | 4.30 | $B_4$ | — |
| | $A_8$ | 4.24 | 3.91 | $B_8$ | — |
| | $C_4$ | 4.12 | 4.36 | $D_4$ | — |
| | $C_8$ | 3.82 | 4.02 | $D_8$ | — |
| | $E_4$ | 4.42 | 4.27 | $F_4$ | — |
| | $E_8$ | 4.33 | 4.11 | $F_8$ | — |
| b) Between wheat and soy flour at each level | Mean scores of wheat (4.80) significantly different from mean score of each of soy samples.** | | | | |
| c) Between levels within a type | $(A+B)_4$ | 4.28 | 4.06 | $(A+B)_8$ | — |
| | $(C+D)_4$ | 4.25 | 3.95 | $(C+D)_8$ | ** |
| | $(E+F)_4$ | 4.34 | 4.21 | $(E+F)_8$ | — |
| d) Between types within and between levels | $(A+B)_4$ | 4.28 | 4.25 | $(C+D)_4$ | — |
| | $(A+B)_4$ | 4.28 | 4.34 | $(E+F)_4$ | — |
| | $(C+D)_4$ | 4.25 | 4.34 | $(E+F)_4$ | — |
| | $(A+B)_4$ | 4.28 | 4.21 | $(E+F)_8$ | — |
| | $(A+B)_8$ | 4.06 | 3.95 | $(C+D)_8$ | — |
| | $(A+B)_8$ | 4.06 | 4.21 | $(E+F)_8$ | — |
| | $(C+D)_8$ | 3.95 | 4.21 | $(E+F)_8$ | * |
| | $(E+F)_4$ | 4.34 | 4.06 | $(A+B)_8$ | * |

— Not significant.
* Significant at 5% level.
** Significant at 1% level, or highly significant.

whereas the 4% and 8% levels were not significantly different for the hexane and ethanol soy flours. When comparisons were made between types of soy flour within and between levels (Table IIId) it was found that the 4% and 8% levels of ethanol-extracted soy flour (E + F) were preferred to the 8% levels of hexane (A + B) and full-fat (C + D). There were no other significant differences.

*Acceptability of Bread.* The scores for acceptability of bread (Table IV) were slightly higher than those for desirability of flavor, indicating that factors other than flavor were considered in scoring for

acceptability. The acceptability scores, nevertheless, were very similar to those for desirability of flavor in that the wheat sample was significantly higher than any of the soy samples, the 4% level of full-fat was significantly higher than the 8% level, and the 4% and 8% levels of ethanol-extracted soy flour were preferred to the 8% levels of hexane and full-fat soy flours, respectively.

## Discussion

The baking studies reported herein indicate that bread of excellent loaf volume and crumb grain may be made from hard winter wheat flour and soy flour blends including up to 8% of the latter. To secure these desirable results, however, it is necessary to use larger quantities of potassium bromate in the baking formula than is customary. These results, together with the high water absorption requirements and buffering properties noted for the soy flours in blends with the hard winter wheat flour, are in agreement with those previously reported by Finney (3) for blends of hard spring wheat flour and soy flour.

Although excellent bread from the standpoint of loaf volume and crumb grain was made from the wheat-soy flour blends, these palatability studies indicated that the wheat bread had more desirable flavor than that baked from soy and wheat flour blends and that the bread made with 8% of full-fat soy flour was less desirable in flavor than that made with 4% of full-fat or with 8% of ethanol-extracted soy flour. These studies also indicated that full-fat soy flour is more easily detected than oil-free flours, particularly if as much as 8% is used in bread, and that 8% of soy flour, either oil-free or full-fat, is more easily detected in bread than 4%.

The flour used in these studies contained more protein than the average flour used by commercial bakers. Thus all the conclusions do not necessarily apply to results which might be obtained in commercial practice with flours containing appreciably less protein.

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# PRODUCTION OF MOLD AMYLASES IN SUBMERGED CULTURE

## II. FACTORS AFFECTING THE PRODUCTION OF ALPHA-AMYLASE AND MALTASE BY CERTAIN ASPERGILLI[1, 2]

HENRY M. TSUCHIYA, JULIAN CORMAN, and HAROLD J. KOEPSELL

**ABSTRACT**

The deleterious effect of low terminal pH in mold cultures on the yield of fungal alpha-amylase, previously reported by various investigators, appears to be due to the inactivation of the enzyme at low pH. Tests showed that fungal alpha-amylase becomes increasingly labile at pH values below 4.5. On the other hand, the stability of fungal maltase is fairly constant over a pH range of 4.2 to 7.25.

In the absence of calcium carbonate, the terminal pH of cultures of *Aspergillus niger* NRRL 337 can be controlled by adjustment of the concentrations of medium ingredients, namely, distillers' thin stillage solids derived from the alcohol fermentation of corn mash and corn meal. Increasing the thin stillage solids content of the medium results in the rise of terminal pH with a consequent increase in yield of alpha-amylase. Increasing the corn meal content of the medium results in a lowering of terminal pH. It also results in an increase in yield of maltase. By adjusting the concentrations of distillers' thin stillage solids and corn meal in the medium, it is possible to control to some degree, the yields of both alpha-amylase and maltase.

Incorporation of calcium carbonate in the medium in concentrations, previously recommended, results in a lowering in the yield of maltase. However, calcium chloride does not exhibit this deleterious effect. Inasmuch as the terminal pH can be controlled in the absence of calcium carbonate by using the proper amounts of thin stillage solids, it is recommended that calcium carbonate be eliminated from the medium used for production of fungal amylase.

Although variation in concentrations of distillers' thin stillage and corn meal in the medium affect the production of alpha-amylase and maltase in cultures of *Aspergillus niger* NRRL 330 and *A. oryzae* NRRL 458, the effect is not as marked as with carbohydrase production by *A. niger* NRRL 337.

The preparation of "fungi diastase" by the submerged culture propagation of *Aspergillus oryzae* in distillers' thin stillage and the utilization of such liquors as the converting agent in distillery operation was proposed by Woolner and Lassloffy as early as 1909 (13). Until recently, however, little or no interest has been displayed in this country in the replacement of distillers' malt by fungal amylases

[1] Manuscript received January 2, 1950.

[2] Contribution from Northern Regional Research Laboratory, Peoria, Illinois. One of the laboratories of the Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, U. S. Department of Agriculture.

produced in submerged culture. In order to obviate the shortcomings inherent in the Amylo process, Erb and Hildebrandt (3) modified the process by supplementing the mold amylase produced in submerged culture of *Rhizopus delemar* with a small amount of malt.

The complete replacement of malt with culture liquors of *Aspergillus niger* NRRL 337 produced by submerged culture processes was first reported by Van Lanen and LeMense (12). Since this communication, Adams *et al.* (1), Erb *et al.* (4) and the Northern Regional Laboratory investigators (5, 2, 11) have published their findings with *A. niger* NRRL 337. The culture liquor of this mold contains at least two carbohydrases, alpha-amylase and maltase. The former is responsible for the dextrinization of starch. Schwimmer (9) and Corman and Langlykke (2) demonstrated the significant role the latter plays in the production of fermentable sugars from starch. Although the possible presence of at least one other carbohydrase has been detected by Tsuchiya, Montgomery and Corman (11), this report is concerned with the factors affecting the yields of alpha-amylase and maltase in submerged culture.

## Material and Methods

*Production of Fungal Amylase Preparations.* Stock cultures of molds were maintained on a medium containing distillers' thin stillage derived from the alcohol fermentation of a corn mash (5% on solids basis or 1.5% on protein basis), soluble starch (2%), and agar (2%). After such stock cultures had sporulated, they were stored at 4°C.

The inoculum was prepared in the following manner: A 24-hr. slant culture was first prepared on the same medium as that used for stock cultures. A loopful of mold was then transferred to 100 ml. of the same medium, less agar, contained in 500 ml. Erlenmeyer flask. The flask was aerated on a reciprocal shaker for 24 hr.

Five ml. of the 24-hr. shake flask culture were used to inoculate 200 ml. of the fungal amylase production medium in 1 liter Erlenmeyer flasks containing variable amounts of corn meal and distillers' thin stillage as the sole ingredients. In some experiments calcium carbonate was also used. The exact medium composition will be given in detail later. The culture was aerated for 6 days at 30°C. on a reciprocal shaker. The mold mycelium was filtered off and the filtrate tested for alpha-amylase and maltase activity.

*Alpha-amylase Activity.* Alpha-amylase was determined by the Olson, Evans, and Dickson (7) modification of the Sandstedt, Kneen, and Blish (8) procedure. A unit of alpha-amylase is that amount of enzyme which dextrinizes 1 g. of beta-amylase treated starch in 1 hr. at 20°C.

*Maltase Activity.* A unit of maltase is that amount of enzyme which hydrolyzes 1 mg. of maltose monohydrate in 1 hr. at 30°C. The method depends on the observation that an increase of 78% in reducing power is obtained when maltose monohydrate is hydrolyzed to glucose under conditions given below. There is a stoichiometric relationship between the amount of enzyme and hydrolysis rate, within limits, when the rate is calculated from the difference in maltose hydrolyzed at 15 and 120 min.

*Reagents:*

(1) Acetate buffer (pH 4.4) solution, 6.0 *M*. 217 ml. of glacial acetic acid and 183 g. of anhydrous sodium acetate are diluted to one liter with water.

(2) Acetate buffer, 0.3 *M*, maltose substrate, 0.06 *M* solution. 2.35 g. of maltose monohydrate (92% pure as calculated on reducing value; e.g., Eastman Kodak Company product) and 5 ml. of acetate buffer (1), are diluted to 100 ml. with water.

(3) Sulfuric acid solution, 1*N*; sodium hydroxide solution, 1*N*; phenolphthalein indicator.

(4) Reagents for sugar estimation by method of Somogyi (10).

*Determination of Maltase Activity.* 5 ml. of fungal amylase preparation (culture filtrate) and 10 ml. of buffered substrate solution (2), both attempered to 30°C. (86°F.), are mixed in a test tube and held in a water bath at 30°C. After 15 min., a 3 ml. aliquot of the reaction mixture is transferred to a 100 ml. volumetric flask containing 3 ml. of 1 *N* sulfuric acid to stop the maltose hydrolysis by acid inactivation of the enzyme. After 120 min., a second 3 ml. aliquot of the reaction mixture is treated in similar manner.

After the acid inactivation of enzyme for 10 min., the acidified reaction mixtures are adjusted to the phenolphthalein end point with 1 *N* sodium hydroxide solution and made up to 100 ml. with water. 5 ml. aliquots are taken for analyses for reducing value (R.V.) by the method of Somogyi using the 20 min. heating period. By this procedure, the R.V. of the reaction mixture after 15 and 120 min. hydrolyses are obtained. The R.V. are measures of the glucose produced, the residual maltose, and the reducing sugars originally present in the enzyme preparation.

*Calculation:*

$a$ = R.V. of 15 min. reaction mixture.

$b$ = R.V. of 120 min. reaction mixture.

$$\frac{(b-a)}{0.78} \times (\text{glucose equivalent of } Na_2S_2O_3 \text{ solution} \times 1.78) \times 20 \times \frac{60}{105}$$

= mg. maltose hydrolyzed per ml. of enzyme preparation per hr.

*Precautions:*

(1) Hydrolysis rate values should be between 2 and 10 mg. maltose hydrolyzed per ml. enzyme preparation per hr. to be acceptable. Values in higher range are preferred.

(2) A pH of 4.4 must prevail in reaction mixture. Fungal amylase preparations highly buffered at pH values other than 4.4. must be adjusted to approximately this point before test.

(3) The copper reagent used in sugar analyses should be measured with a pipet rather than a buret.

## Results and Discussion

*Effect of pH on Enzymes.* LeMense and his associates (5) showed that yields of alpha-amylase are lowered in cultures with low terminal pH. They recommended that calcium carbonate be incorporated as a buffering agent in the medium used for the propagation of molds for maximal yield of this enzyme. Inasmuch as the yield is a function of both stability and formation of the enzyme, the effect of pH on the stability of mold alpha-amylase and maltase of *A. niger* NRRL 337 was first studied. Culture filtrates were adjusted to various pH values between 4.2 and 7.25 with buffers and held at 30°C. for 18 hr. The data in Table I indicate that alpha-amylase was stable over a pH range of 4.75 to 7.25. However, some destruction of the enzyme occurred at pH 4.45 and considerable inactivation took place at 4.2. On the other hand, maltase was stable over the entire range tested. If alpha-amylase were stable over the entire range, such a fact would have suggested that the enzyme is not produced in culture of low pH. However, since it was inactivated at low pH values, the possibility existed that it may be produced and subsequently inactivated under acid conditions.

TABLE I

EFFECT OF pH ON STABILITY OF CARBOHYDRASES OF *Aspergillus niger* NRRL 337

| Culture Filtrate | Alpha-amylase | Maltase |
|---|---|---|
| *pH* | *units/ml.* | *units/ml.* |
| 4.2 | 2.3 | 2.9 |
| 4.45 | 6.7 | 2.7 |
| 4.75 | 8.4 | 2.9 |
| 5.7 | 8.6 | 2.8 |
| 6.5 | 8.6 | 2.9 |
| 7.25 | 8.4 | 2.7 |

Temperature: 30°C.
Time: 18 hr.
Original alpha-amylase content: 8.6 units/ml.
Original maltase content: 2.8 units/ml.

TABLE II

EFFECT OF INITIAL pH ON YIELDS OF CARBOHYDRASES IN CULTURES OF *Aspergillus niger* NRRL 337

| pH | Alpha-amylase | Maltase |
|---|---|---|
| | *units/ml.* | *units/ml.* |
| 3.7 | 3.5 | 12.3 |
| 4.2 | 8.9 | 12.7 |
| 4.5 | 11.4 | 12.1 |
| 5.0 | 11.9 | 12.8 |
| 5.7 | 12.1 | 12.8 |
| 6.25 | 8.6 | 12.5 |

The effect of initial pH of the medium on enzyme yield was studied by varying the pH with 1 *N* sodium hydroxide or hydrochloric acid over the range of 3.7 to 6.25. *A. niger* NRRL 337 was grown in a 2% corn and 5% thin stillage solids medium to which calcium carbonate was not added. The results shown in Table II indicate that yields of alpha-amylase decreased when the initial pH was lowered below 4.5 or raised above 5.7. Since the enzyme is stable at pH values up to 7.25, the results together with those from the previous experiment indicate that alpha-amylase production is inhibited at the higher pH. On the other hand, maltase yields were constant over the entire range tested.

*Effect of Medium Composition on Terminal pH and Enzyme Yields.* Inasmuch as the results were at variance with those reported by LeMense and his associates (5) and also by Erb and his coworkers (4), namely, that calcium carbonate must be incorporated in the medium for adequate enzyme production, this phenomenon was examined more closely. It appeared that the pH had been controlled by the composition of our medium. Since it is frequently possible to control the pH in growing cultures by adjusting the amounts of carbon and

TABLE III

EFFECT OF MEDIUM COMPOSITION ON TERMINAL pH OF CULTURES OF *Aspergillus niger* NRRL 337

| Thin Stillage Solids | Corn % | | | |
|---|---|---|---|---|
| | 2 | 5 | 7 | 10 |
| | Terminal pH of culture filtrates | | | |
| % | | | | |
| 1 | 3.5 | 3.2 | 3.3 | 3.5 |
| 3 | 4.7 | 4.0 | 3.9 | 3.9 |
| 5 | 4.5 | 4.2 | 4.1 | 4.0 |
| 7 | 4.6 | 4.3 | 4.3 | 4.1 |

nitrogen sources (e.g., increasing the former lowers the pH whereas increasing the latter raises the pH), the effect of varying the corn and thin stillage solids contents of the medium was tested. The corn concentration was varied from 2 to 10% and the thin stillage solids level from 1 to 7%. The initial pH on the various media was adjusted to 5.2 with 1 *N* sodium hydroxide after sterilization. The effect on terminal pH of cultures of *A. niger* NRRL 337 is shown in Table III. As the concentration of corn is increased the terminal pH usually drops, but as the level of thin stillage solids is increased, the pH rises. The terminal pH in cultures containing calcium carbonate is approximately 5.0. This would appear to explain the discrepancy found between the data of LeMense and his associates (5) and ours. Presumably, they worked with thin stillage in which the solids content was often low, and therefore, in the absence of calcium carbonate, the pH dropped to low values although low levels of corn were used. This resulted in a destruction of alpha-amylase. In the present experiments the thin stillage solids level of the mold culture medium was controlled by dilution of thin stillage sirup. Thus, by controlling the concentration of thin stillage solids contents, we controlled the terminal pH.

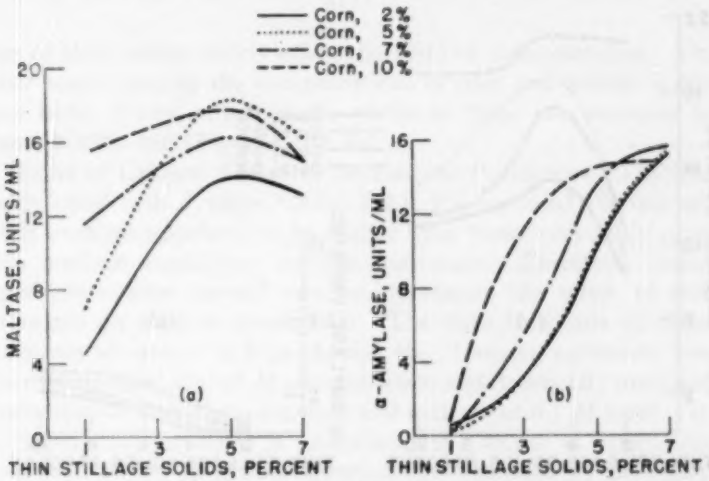


FIG. 1. Effect of medium composition on maltase and alpha-amylase production of *Aspergillus niger* NRRL 337.

Perhaps more interesting than the effect of corn and thin stillage solids levels on the terminal pH of cultures was their effect on yields of maltase and alpha-amylase with *A. niger* NRRL 337. The yield of maltase at low levels of thin stillage solids is dependent upon the

concentration of corn as indicated in Fig. 1a. Although the use of higher concentrations of corn generally results in both the lowering of pH and the improvement in yields of maltase, evidence at hand indicates that factors other than pH are also involved. The beneficial effect of corn on the elaboration of fungal amylases has also been reported by Erb and his associates (4). As the stillage solids concentration is increased to optimal level, the effect of corn is minimized to some extent. As can be seen from Fig. 1b, thin stillage solids exert a marked effect on the yield of alpha-amylase. At 1% level, only trace amounts of this enzyme are elaborated, even in medium containing 10% corn. The low terminal pH values of these cultures probably destroyed this enzyme. As the thin stillage solids concentration is

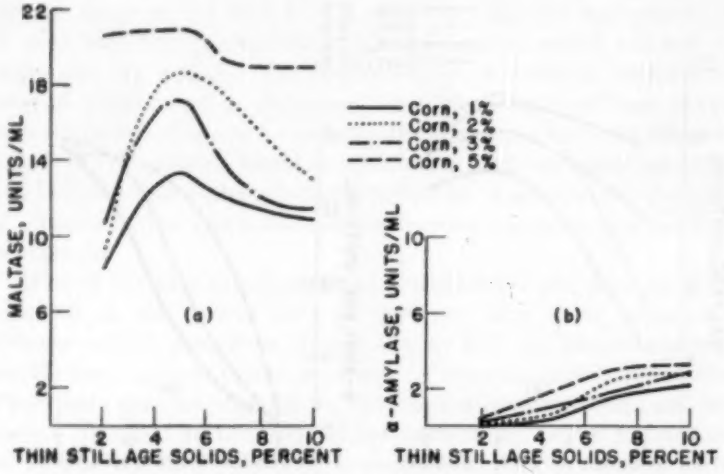


FIG. 2. Effect of medium composition of maltase and alpha-amylase production of *Aspergillus niger* NRRL 330.

increased, the terminal pH rises with a corresponding increase in the alpha-amylase content. That some other factor is also operative in the yield of alpha-amylase after the pH reaches 3.9, is seen by the fact that yields increase faster in media containing the higher concentrations of corn.

To see if enzyme yields could be controlled as readily with other organisms, this experiment was repeated with *A. niger* NRRL 330, a high maltase and low alpha-amylase producing mold, and *A. oryzae* NRRL 458, a low maltase and moderate alpha-amylase producing organism. Essentially the same effects are obtained with *A. niger* NRRL 330 (Figs. 2a and 2b). Increasing the level of corn to an optimal point raises the yield of maltase and increasing the concentra-

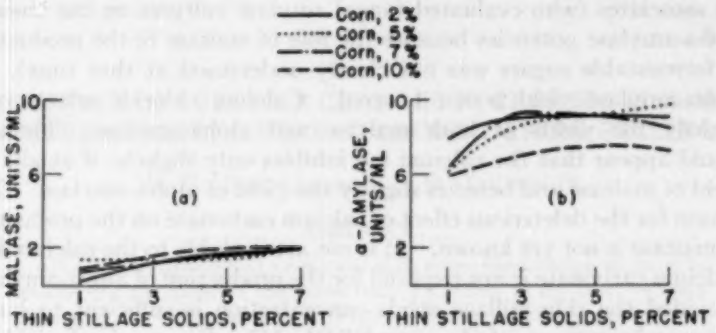


FIG. 3. Effect of medium composition on maltase and alpha-amylase production of *Aspergillus oryzae* NRRL 458.

tion of thin stillage solids raises the yield of alpha-amylase. On the other hand, varying the concentrations of corn and stillage solids has very little, if any, effect on the yields of these two enzymes by *A. oryzae* NRRL 458 (Figs. 3a and 3b).

*Effect of Calcium Carbonate on Enzyme Production.* The maltase yields found with *A. niger* NRRL 337 in 2% corn and 5% thin stillage solids medium appeared to be higher than those previously obtained with medium containing calcium carbonate. Therefore, controlled experiments were carried out to investigate the effect of calcium carbonate on enzyme production. The data from one of these experiments are shown in Figs. 4a and 4b. Calcium carbonate lowered the maltase yield at 0.05 *M* concentration and drastically inhibited the production of both alpha-amylase and maltase at 0.1 *M* level. It will be noted that at 0.05 *M* concentration, which is approximately equivalent to the 0.5% concentration recommended by LeMense and

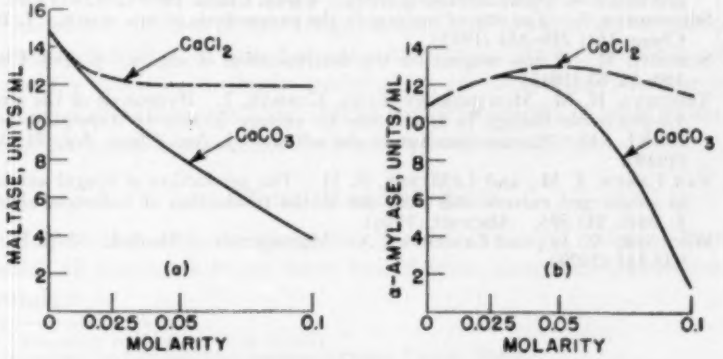


FIG. 4. Effect of calcium carbonate and calcium chloride on maltase and alpha-amylase production of *Aspergillus niger* NRRL 337.

his associates (who evaluated fungal amylase cultures on the basis of alpha-amylase potencies because the role of maltase in the production of fermentable sugars was not clearly understood at that time), the alpha-amylase yield is not lowered. Calcium chloride affects only slightly the yields of both maltase and alpha-amylase. Thus, it would appear that the calcium ion inhibits only slightly, if at all, the yield of maltase and benefits slightly the yield of alpha-amylase. The reason for the deleterious effect of calcium carbonate on the production of maltase is not yet known. It is not attributable to the calcium ion. Calcium carbonate is not required for the production of alpha-amylase, provided the thin stillage solids concentration is sufficient to buffer adequately cultures of *A. niger* NRRL 337. For maximal yields of maltase, calcium carbonate should be eliminated from the medium. Pilot-plant studies which will be reported elsewhere bear out the conclusions reached from these experiments.

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# THE EFFECT OF CRUST ON CHANGES IN CRUMBLINESS AND COMPRESSIBILITY OF BREAD CRUMB DURING STALING[1,2]

W. B. BRADLEY and J. B. THOMPSON

**ABSTRACT**

A series of experiments were made with normal and decrusted bread to determine if the moisture migration from the crumb to the crust had any effect upon the compressibility or crumbliness tests which are employed to follow the progress of crumb staling. Although moisture determinations revealed a loss of moisture from the crumb of the intact loaves amounting to 8% of the crumb moisture and a relatively constant moisture in the decrusted crumb during the course of the experiments, the rate and extent of changes in crumbliness and compressibility of the intact and decrusted bread were not appreciably different.

The changes in the crust of bread which occur during staling have generally been recognized as being caused by absorption of moisture. The sources of this moisture are known to be the internal crumb of the bread and, perhaps in some cases of high humidity, the atmosphere. In the case of modern-wrapped bread, the crumb is the more important source of moisture.

Geddes and his associates (1) have shown that appreciable quantities of water migrate from the crumb to the crust of bread which has been sealed in metal cans to prevent loss of moisture to the atmosphere. They reported the moisture content of the crust to increase from 15.4% for the fresh-baked loaf to 27.3% after six days of storage. Simultaneously, the moisture content of the central crumb decreased from 45.1% in the fresh bread to 37.0% in that stored for six days.

Moisture changes of this magnitude might be expected to decrease the compressibility of bread crumb and increase its crumbliness. Experiments were accordingly undertaken to determine whether this migration of moisture is a significant factor in results of such measurements on bread crumb after various intervals of storage.

## Materials and Methods

*Preparation and Storage of the Bread.* For each series of experiments, 18 loaves of bread were baked from dough of the following formula:

[1] Manuscript received December 15, 1949.
[2] Contribution from American Institute of Baking, Chicago, Illinois.
The study on which this article is based was made by the American Institute of Baking, under contract with the U. S. Department of Agriculture. The work was done under authority of the Research and Marketing Act.

| | % |
|---|---|
| Flour | 100 |
| Sugar | 4 |
| Nonfat Dry Milk Solids | 3 |
| Shortening | 3 |
| Salt | 2 |
| Yeast | 2 |
| Arkady | 0.25 |
| Calcium Propionate | 1.0 |
| Water | Variable |

The results of preliminary studies with bread containing 0.3% of calcium propionate indicated a decrease in the moisture content of the crumb from the intact loaves of about 4% and an increase in crust moisture of about 9% in 96 hr. The crustless bread, however, was found to have a significantly higher moisture content than initially after the first 24 hr. due to growth of mold. Precautions were therefore necessary to prevent microbiological contamination of the samples used. Consequently, the calcium propionate in the bread formula was increased to 1% and a technique of preparing the decrusted bread was developed.

As soon as the bread cooled (about 1½ hrs. after removal from the oven), eight of the loaves were carefully stripped of the outer 1 cm. of crust and outer crumb by means of a thin-bladed knife, honed to razor sharpness, to minimize the possibility of mechanical damage to the fresh crumb. Handling of the crumb was avoided in an attempt to minimize its contamination with micro-organisms.

The crumb-sections and eight intact loaves were immediately placed in individual heat-sterilized, press-top tin containers and stored in a constant temperature cabinet at 35°C. The remaining two loaves were used immediately for moisture, compressibility, and crumbliness determinations.

*Moisture Determinations.* Moisture determinations were made daily upon the center crumb from the entire and the crustless loaves and on the crust from the intact loaves. A section of center crumb weighing about 30 g. was weighed to the nearest centigram and air-dried overnight. The dehydration was then completed in a vacuum oven.

The moisture of the crust from the intact loaf was determined after the removal of as much crumb as possible. These determinations represented the moisture content of only the outer 3–4 mm. of crust which was composed essentially of the highly caramelized material.

*Compressibility Determinations.* Compressibility measurements were made upon three slices from each of two loaves of the bread. The crust was cut from the intact bread, and all slices were trimmed to provide sections 2.5 × 5 × 5 cm. in size.

The compressibility of these sections was determined by means of a penetrometer of the type specified by the American Society of Testing Materials for use with petroleum products, as reported by Sumner and Thompson (2). The penetration cone was replaced by a disk 3 cm. in diameter. The surface of the crumb-section was raised to this plate, which was then released to compress the crumb under a total load of 215 g. for 10 sec. The average of six such measurements was taken as the compressibility value.

*Crumbliness Determinations.* The crumbliness of the bread crumb was determined by a variation of a procedure reported by Bice and Geddes (1) and previously used by Sumner and Thompson (2).

Fifty grams of 12 mm. cubes were cut with the aid of a miter box and placed in a U. S. No. 4 sieve equipped with a catch pan and lid. A sheet metal disk 18 cm. in diameter and weighing 56 g. was placed on top of the cubes and shaken in a Precision Scientific Shaker for 15 min. The sifted crumb was weighed to the nearest centigram and taken as a measurement of crumbliness.

## Results

*Storage at 35°C.* The results of daily moisture, compressibility and crumbliness measurements on intact and crustless loaves stored at 35°C. for four days are presented in Table I. A significant migration of moisture from the crumb to the crust occurred, although its magnitude

TABLE I

MOISTURE, COMPRESSIBILITY, AND CRUMBLINESS CHANGES IN BREADS Stored at 35°C.

| Age | Bread with Crust | | | | | | Bread without Crust | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Moisture | | | | Compressibility | Crumbliness | Moisture | | Compressibility | Crumbliness |
| | Crumb | | Crust | | | | Crumb | | | |
| *hrs.* | *Total %* | *Loss %* | *Total %* | *Gain %* | *mm.* | *g.* | *Total %* | *Loss %* | *mm.* | *g.* |
| 1.5 | 44.9 | 0.0 | 25.1 | 0.0 | 12.1 | 0.16 | 44.9 | 0.0 | 12.1 | 0.16 |
| 24 | 43.6 | 2.9 | 30.5 | 21.5 | 8.7 | 0.74 | 44.7 | 0.4 | 8.2 | 0.85 |
| 48 | 43.0 | 4.2 | 31.7 | 26.3 | 5.6 | 1.18 | 44.1 | 1.8 | 6.6 | 1.02 |
| 72 | 42.4 | 5.6 | 32.7 | 30.2 | 4.5 | 1.76 | 44.4 | 1.1 | 4.8 | 1.62 |
| 96 | 41.4 | 7.8 | 32.9 | 31.0 | 4.2 | 2.79 | 44.8 | 0.2 | 4.7 | 4.69 |

was much less than that reported by Bice and Geddes (1). There was no significant change in the moisture content of the decrusted bread during the period of the experiment. In spite of the definite decrease in crumb moisture of the intact loaves, there was no difference between their compressibility and that of the crustless loaves when moisture

TABLE II

MOISTURE, COMPRESSIBILITY, AND CRUMBLINESS CHANGES IN BREAD Stored at 24°C.

| Age | Bread with Crust | | | | | | Bread without Crust | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Moisture | | | | Compressibility | Crumbliness | Moisture | | Compressibility | Crumbliness |
| | Crumb | | Crust | | | | Crumb | | | |
| *hrs.* | *Total* % | *Loss* % | *Total* % | *Gain* % | *mm.* | *g.* | *Total* % | *Loss* % | *mm.* | *g.* |
| 1.5 | 42.9 | 0.0 | 20.0 | 0.0 | 9.1 | 0.53 | 42.9 | 0.0 | 9.1 | 0.53 |
| 24 | 40.5 | 5.6 | 25.8 | 29.0 | 4.0 | 1.77 | 42.5 | 0.9 | 3.9 | 2.05 |
| 48 | 39.5 | 7.9 | 27.5 | 37.5 | 1.9 | 7.10 | 42.5 | 0.9 | 2.0 | 8.42 |
| 72 | 39.5 | 7.9 | 30.0 | 50.0 | 1.9 | 12.49 | 42.3 | 1.4 | 1.7 | 9.62 |
| 96 | 39.4 | 8.2 | 30.7 | 53.5 | 1.5 | 15.71 | 42.6 | 0.7 | 1.5 | 13.16 |

migration was prevented. The crumbliness values were also remarkably similar except on the last day of measurement.

*Storage at 24°C.* The data obtained in a similar study on breads stored at 24°C. are presented in Table II. The moisture content of the crumb from the intact loaves was found to decrease at the same rate as occurred in that stored at 35°C., though there was a slight decrease in the rate of moisture increase in the crust. The compressibility and crumbliness values of the crustless and intact bread were not appreciably different at each time interval.

## Discussion

The results of these experiments indicate that the normal loss of moisture from bread crumb due to migration of water to the crust has little or no influence upon values obtained by applying the compressibility and crumbliness tests for the evaluation of staling. These two physical tests would appear to measure the effects of physical or chemical changes in the bread crumb which are known as staling and are not related to, or affected by, gross moisture migrations from one part of the loaf to another. In general, the migration of moisture from the crumb to the crust of bread caused an increase of as much as 50% of the initial moisture content of the latter in a 96-hr. period. During a like interval, a migration of approximately 8% of the center crumb moisture was found. In the case of bread from which the crust had been removed and which was stored to preclude evaporation, there was no change in crumb moisture.

Boutroux (3) was one of the first to theorize concerning the cause of bread staling. He postulated that during the staling of bread, moisture migration from crumb to crust caused the crumb to become dry and firm and the crust to become soft and leathery. He believed that

heating refreshened bread by driving the water from the crust back into the crumb, thus softening the crumb and producing a fresh, crisp crust. His theory has long since been discarded because of the inability of subsequent investigators to find a supersaturated solution of amylodextrin in fresh bread, which was also a hypothesis included in his theory.

Data presented in this paper add further to the denial of the validity of Boutroux's theory. If either crumbliness or compressibility of crumb is a measure of bread staling, then staling is in no way dependent upon migration of water from the crumb to the crust, because the rate and extent of change of crumbliness and compressibility are as great in the crumb of bread in which migration of water is prevented as in the crumb of an intact loaf.

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## CHARACTERIZATION OF WHEAT GLUTEN
## II. AMINO ACID COMPOSITION[1, 2]

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### ABSTRACT

The amino-acid compositions of glutens prepared by ordinary washing procedures from 17 different flours were determined principally by microbiological assay methods. The compositions of the glutens were found to be essentially uniform, despite the wide range in type and source of the wheats and in the protein contents and baking characteristics of the flours from which the glutens were obtained. The average values, which are believed to be minimal because of the considerable amount of carbohydrate present during acid hydrolysis, are: ammonia, 4.5; alanine, 2.2; arginine, 4.7; aspartic acid, 3.7; cystine plus cysteine, 1.9; glutamic acid, 35.5; glycine, 3.5; histidine, 2.3; isoleucine, 4.6; leucine, 7.6; lysine, 1.8; methionine, 1.9; phenylalanine, 5.4; proline, 12.7; serine, 4.7; threonine, 2.6; tryptophan, 1.1; tyrosine, 3.1; valine, 4.7; as per cent of protein of theoretical nitrogen content of 17.5%.

[1] Manuscript received January 3, 1950. Paper I of this series was published in Cereal Chem. **24**: 407–414 (1947).
[2] Contribution from Western Regional Research Laboratory, Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, U. S. Department of Agricu ture, Albany, California.

Despite a number of attempts, no one has yet succeeded in finding a complete explanation for the differences in baking behavior of different flours. It is known that the *amount* of protein present is an important factor, yet flours with *equal* protein content often differ markedly in baking quality. As part of a systematic study of the factors responsible for these differences, we have now analyzed glutens from 17 flours of widely varying characteristics for their amino acid composition. No appreciable differences were found. Nevertheless, the analyses are presented, inasmuch as they are probably more nearly complete and accurate than those hitherto available.

## Materials and Methods

The flours used were straight-grade, unbleached flours experimentally milled from samples of pure varieties. The series included wheats of all major types and showed a wide range of protein content and baking behavior. Loaf volumes obtained in this Laboratory ranged from 435 cc. to 890 cc., using the baking formula of Finney and Barmore (5) with optimum bromate and mixing time.

Glutens were prepared from the flours by hand-washing doughs with the 0.1%, pH 6.8 phosphate buffer of Dill and Alsberg (4). The glutens contained, on the average, 88% of the flour nitrogen and 76% of the flour sulfur. Hydrolyzates for amino acid analysis were prepared by refluxing 2.5 g. of lyophilized gluten in 50 ml. of 6 *N* hydrochloric acid for 18 hr. Sufficient starch was added to each sample before hydrolysis so that all samples contained 12% nitrogen.[3] Most of the hydrochloric acid was removed by repeated concentration of the hydrolyzate under vacuum; after dilution to volume (100 ml.), the hydrolyzates were allowed to stand overnight before removal of the insoluble humin by filtration. Aliquots were neutralized for microbiological assay.

Tryptophan was determined in unhydrolyzed gluten by the method of Horn and Jones (6). Cystine-plus-cysteine values were obtained on hydrolyzates by a method (10) based on that of Vassel (14). Values for the remaining amino acids were obtained by microbiological assay of hydrolyzates with the following organisms: *Leuconostoc mesenteroides* P-60 (ATCC 8042) (arginine, aspartic acid, glycine, histidine, lysine, methionine, phenylalanine, proline, serine, and tyrosine); *Lactobacillus arabinosus* 17-5 (ATCC 8014) (glutamic acid, isoleucine, leucine, methionine, and valine); *Leuconostoc citrovorum* (ATCC 8081) (alanine and serine); *Streptococcus faecalis* R (ATCC 8043) (threonine).

[3] The different samples of washed glutens contained varying quantities of residual starch. The addition of starch to constant nitrogen content was designed to minimize variations in amino acid composition that might have resulted from destruction of amino acids during hydrolysis by reaction with carbohydrate break-down products.

## TABLE I

AMINO ACID CONTENTS OF WHEAT GLUTENS FROM FLOURS OF VARYING TYPES
Amino Acid Values Expressed as Percentage of the Protein of Assumed 17.5% Nitrogen Content

| Wheat Variety | Type | Source | Protein in flour[1] (N × 5.7) | Ammonia | Alanine | Arginine | Aspartic Acid | Cystine plus Cysteine | Glutamic Acid | Glycine | Histidine | Isoleucine |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | % | % | % | % | % | % | % | % | % | % |
| Hymar | W club | Wash. | 5.7 | 4.2 | — | 5.0 | 4.1 | 2.0 | 34.9 | 3.2 | 2.3 | 4.6 |
| Yorkwin | WW | Mich. | 7.6 | 4.3 | — | 4.9 | 3.9 | 2.0 | 34.6 | 3.2 | 2.3 | 4.5 |
| Rex | WW | Wash. | 7.8 | 4.3 | — | 5.2 | 3.6 | 1.8 | 35.2 | 3.5 | 2.4 | 4.4 |
| Purkof | SRW | Ind. | 8.7 | 4.4 | — | 4.8 | 3.6 | 1.9 | 34.8 | 3.4 | 2.2 | 4.4 |
| Tenmarq | HRW | Kans. | 8.8 | 4.5 | — | 4.8 | 3.8 | 2.0 | 34.3 | 3.9 | 2.3 | 4.5 |
| Hymar | W club | Wash. | 9.0 | 4.6 | — | 4.5 | 3.6 | 1.9 | 34.5 | 3.2 | 2.3 | 4.7 |
| Goens | SRW | Ind. | 9.1 | 4.4 | — | 4.6 | 3.5 | 1.9 | 35.5 | 3.4 | 2.2 | 4.4 |
| Baart | WS | Wash. | 9.5 | 4.5 | 2.1 | 4.5 | 3.6 | 1.8 | 34.7 | 3.3 | 2.2 | 4.4 |
| Chiefkan | HRW | Kans. | 9.6 | 4.4 | 2.3 | 4.8 | 3.9 | 1.9 | 34.9 | 3.9 | 2.4 | 4.7 |
| Comanche | HRW | Kans. | 9.7 | 4.6 | — | 4.8 | 3.8 | 1.9 | 34.9 | 3.8 | 2.4 | 4.7 |
| Turkey | HRW | Kans. | 10.2 | 4.4 | — | 4.7 | 3.9 | 2.0 | 36.7 | 3.5 | 2.3 | 4.5 |
| Red Chief | HRW | Kans. | 10.3 | 4.5 | — | 4.8 | 3.9 | 2.1 | 35.1 | 3.4 | 2.3 | 4.6 |
| Pentad | Durum | No. Dak. | 11.6 | 4.5 | 2.3 | 4.8 | 3.8 | 1.8 | 36.2 | 3.2 | 2.4 | 4.8 |
| Premier | HRS | No. Dak. | 11.7 | 4.6 | — | 4.8 | 3.7 | 1.8 | 36.4 | 3.5 | 2.4 | 4.8 |
| Red Chief | HRW | Texas | 12.9 | 4.5 | 2.2 | 4.6 | 3.5 | 2.0 | 36.8 | 3.2 | 2.4 | 4.6 |
| Turkey | HRW | Texas | 13.4 | 4.4 | 2.2 | 4.6 | 3.6 | 1.7 | 37.6 | 3.4 | 2.3 | 4.5 |
| Thatcher | HRS | Mont. | 14.2 | 4.6 | 2.1 | 4.3 | 3.5 | 1.8 | 36.9 | 3.8 | 2.3 | 4.4 |
| Mean | | | | 4.5 | 2.2 | 4.7 | 3.7 | 1.9 | 35.5 | 3.5 | 2.3 | 4.6 |
| Standard Deviation | | | | ±.1 | ±.1 | ±.2 | ±.2 | ±.1 | ±1.0 | ±.3 | ±.1 | ±.1 |

[1] 14% moisture.

TABLE I—*Continued*

| Wheat Variety | Type | Source | Leucine | Lysine | Methionine | Phenyl-alanine | Proline | Serine | Threonine | Trypto-phan | Tyrosine | Valine |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | % | % | % | % | % | % | % | % | % | % |
| Hymar | W club | Wash. | 8.0 | 2.1 | 2.1 | 5.3 | 12.6 | — | 2.6 | 1.1 | 3.1 | 5.0 |
| Yorkwin | WW | Mich. | 8.0 | 2.0 | 2.0 | 5.4 | 13.3 | — | 2.6 | 1.1 | 3.1 | 4.7 |
| Rex | WW | Wash. | 7.6 | 2.1 | 1.9 | 5.1 | 12.9 | — | 2.6 | 1.3 | 3.6 | 4.8 |
| Purkof | SRW | Ind. | 7.2 | 1.9 | 1.7 | 5.0 | 12.4 | — | 2.7 | 1.2 | 3.2 | 4.5 |
| Tenmarq | HRW | Kans. | 7.2 | 1.9 | 1.8 | 5.1 | 11.9 | — | 2.6 | 1.1 | 3.0 | 4.6 |
| Hymar | W club | Wash. | 7.7 | 1.7 | 1.8 | 5.6 | 12.6 | — | 2.6 | 1.1 | 3.1 | 4.7 |
| Goens | SRW | Ind. | 7.0 | 1.7 | 1.8 | 5.1 | 11.7 | — | 2.5 | 1.0 | 2.9 | 4.4 |
| Baart | WS | Wash. | 7.2 | 1.7 | 1.8 | 5.2 | 12.1 | 4.4 | 2.4 | 1.1 | 3.0 | 4.4 |
| Chiefkan | HRW | Kans. | 7.8 | 1.8 | 1.9 | 5.3 | 12.6 | 4.8 | 2.7 | 1.1 | 3.2 | 4.7 |
| Comanche | HRW | Kans. | 8.0 | 1.9 | 1.8 | 5.4 | 12.7 | — | 2.8 | 1.1 | 3.1 | 4.8 |
| Turkey | HRW | Kans. | 8.0 | 1.9 | 2.0 | 5.4 | 13.3 | — | 2.9 | 1.1 | 3.3 | 4.7 |
| Red Chief | HRW | Kans. | 7.8 | 1.8 | 1.9 | 5.2 | 12.7 | — | 2.6 | 1.1 | 3.0 | 4.9 |
| Pentad | Durum | No. Dak. | 7.1 | 1.8 | 1.7 | 5.8 | 12.4 | 4.7 | 2.5 | 1.0 | 2.8 | 4.6 |
| Premier | HRS | No. Dak. | 8.0 | 1.7 | 2.0 | 5.7 | 13.3 | — | 2.4 | 1.1 | 3.1 | 4.9 |
| Red Chief | HRW | Texas | 7.4 | 1.7 | 1.8 | 5.7 | 13.0 | 5.0 | 2.4 | 1.0 | 3.1 | 4.7 |
| Turkey | HRW | Texas | 7.5 | 1.8 | 1.9 | 5.7 | 13.2 | 5.0 | 2.5 | 1.1 | 3.3 | 4.7 |
| Thatcher | HRS | Mont. | 7.5 | 1.7 | 1.7 | 5.7 | 13.5 | 4.5 | 2.4 | 1.1 | 3.0 | 4.6 |
| Mean | | | 7.6 | 1.8 | 1.9 | 5.4 | 12.7 | 4.7 | 2.6 | 1.1 | 3.1 | 4.7 |
| Standard Deviation | | | ±.3 | ±.1 | ±.1 | ±.2 | ±.5 | ±.2 | ±.1 | ±.1 | ±.2 | ±.2 |

[1]14% moisture.

Details regarding the basal media, standards, and procedure will be described elsewhere (7). Amide nitrogen of the glutens was determined by a method previously described (11).

## Results and Discussion

The variety, type, and source of each of the 17 wheats and the amino acid contents of the corresponding glutens (calculated to 17.5% nitrogen[4] are presented in Table I.

The variation in amino acid contents among the glutens is quite small in most instances, although the range of values exceeds 20% of the mean for several of the amino acids. The coefficients of variability (standard deviation expressed as per cent of the mean), however, fall between 2.8 and 7.3%, which indicates that the dispersions of values around the means are quite small generally. Such variations are insignificant in face of the ±10% limit of error often applied to microbiological amino acid assays.

Of particular interest are comparisons between pairs of flours of almost equal protein content but widely different baking characteristics. Two such pairs are: Turkey (Kansas) and Red Chief (Kansas); Comanche and Chiefkan. In each case, the latter of the pair is inferior as shown not only by experience in the baking industry but also confirmed by baking tests both in this Laboratory and elsewhere, with the particular flours. Loaf volumes obtained in this Laboratory for the Turkey (Kansas) and Red Chief (Kansas) flours were 665 cc. and 515 cc., respectively, and 630 cc. and 530 cc. for the Comanche and Chiefkan flours. Casual inspection reveals no trends upward or downward in the amount of any particular amino acid in the better member of the pairs. With Comanche and Chiefkan particularly, the analyses are almost identical.

The general uniformity of composition of the glutens is in agreement with the earlier reports of Blish (1) and Cross and Swain (3), who found no significant variation in amino acid composition among gliadins and glutenins prepared from different types of flour. However, McElroy *et al.* (9) analyzed whole wheats and obtained evidence that significant, although small, differences in lysine, arginine, and valine contents existed among different samples of a single variety (Marquis). These variations, of course, may reflect differences in the non-gluten nitrogenous components. Similarly, some of the variations in the series of analyses shown in Table I may reflect the presence of small amounts of non-gluten proteins. As an example, the slightly low amide nitrogen content of the glutens obtained from the three

[4] The 17.5% nitrogen corresponds to the factor, 5.7, traditionally used by cereal chemists to convert per cent nitrogen in wheat flour to per cent protein.

flours of lowest protein content can possibly be ascribed to the difficulties of washing these glutens uniformly free from the other proteins.

It should be emphasized that the amino acid contents found are minimum values, inasmuch as considerable destruction may have occurred as a result of the presence of the relatively large amounts of carbohydrate[5] substances during acid hydrolysis. Cystine, methionine,[6] tyrosine, tryptophan, and the basic amino acids are affected by such treatment (13, 8). Serine and threonine are known to be partially destroyed even in the absence of carbohydrate (12). Nevertheless,

TABLE II

AVERAGE COMPOSITION OF WHEAT GLUTEN

| Constituent | G. Amino Acid per 100 g. Protein[1] | | Moles Amino Acid per $10^5$ g. Protein[1] | Amino Acid Nitrogen of Total Nitrogen |
|---|---|---|---|---|
| | Found | Literature[2] | | % |
| Alanine | 2.2 | 5.5 | 25 | 2.0 |
| Ammonia | 4.5 | 4.5 | 264 | 21.2 |
| Arginine | 4.7 | 4.3 | 27 | 8.6 |
| Aspartic acid | 3.7 | — | 28 | 2.2 |
| Cystine | 1.9 | 1.9 | 8 | 1.3 |
| Glutamic acid | 35.5 | 36.0 | 241 | 19.3 |
| Glycine | 3.5 | — | 47 | 3.7 |
| Histidine | 2.3 | 2.4 | 15 | 3.6 |
| Isoleucine | 4.6 | —[3] | 35 | 2.8 |
| Leucine | 7.6 | —[3] | 57 | 4.6 |
| Lysine | 1.8 | 2.1 | 12 | 2.0 |
| Methionine | 1.9 | 3.3 | 13 | 1.0 |
| Phenylalanine | 5.4 | 2.0 | 33 | 2.6 |
| Proline | 12.7 | 11.0 | 110 | 8.8 |
| Serine | 4.7 | — | 45 | 3.6 |
| Threonine | 2.6 | 2.5 | 21 | 1.7 |
| Tryptophan | 1.1 | 1.1 | 5 | 0.9 |
| Tyrosine | 3.1 | 4.2 | 17 | 1.4 |
| Valine | 4.7 | 3.0 | 40 | 3.2 |
| Total | 108.5 | | 779[4] | 94.5 |

[1] Computed for a theoretical protein containing 17.5% nitrogen.
[2] Values cited by Blish, Advances in Protein Chemistry, II: 337-359 (1945).
[3] The literature value for the sum of leucine and isoleucine is 6.0.
[4] Number of moles of ammonia omitted from total.

approximately 95% of the total nitrogen is accounted for, as shown in Table II, without application of any correction to the analytical data. It can be inferred that such corrections, if known, would increase the total nitrogen accounted for to near 100%, and that no other amino acids were present, except in trace amounts.

No analyses were made for hydroxylysine or hydroxyproline. Rees (12) showed that hydroxylysine was absent from gliadin. Since

[5] Addition of starch to a gliadin preparation (nitrogen content, 17.0%) in amounts sufficient to reduce the nitrogen content to 16.0, 14.0, and 12.0% prior to hydrolysis caused apparent losses in cystine-plus-cysteine of 4, 10, and 18%, respectively. Results with methionine were approximately the same. The total cystine-plus-methionine values reported in the tables account for an average of 81% of the total gluten sulfur.

there are no satisfactory methods for determining hydroxyproline, its possible presence was not investigated. However, the occurrence of these amino acids in proteins is believed to be limited to those related to collagen.

The mean values for many of the amino acids agree well with those compiled by Blish (2) (Table II). Our values for phenylalanine, leucine-plus-isoleucine, and valine are considerably higher and for alanine,[6] methionine, and tyrosine significantly lower than those previously available.

**Acknowledgments**

We are indebted to Mr. Roy K. Durham and the Millers National Federation for 13 of the flour samples used in this study. These samples were milled by Pillsbury Mills, Inc., Minneapolis. Baking data were supplied by Mr. G. Moen, General Mills, Inc., Minneapolis. Three samples of flour with baking data were furnished by the Bureau of Plant Industry, Soils, and Agricultural Engineering, Manhattan, Kansas. The remaining sample of flour was obtained through the courtesy of the Oregon Wheat Commission.

The authors wish also to acknowledge the careful technical assistance of Mrs. F. C. Marsh and Mrs. P. A. Thompson with the microbiological assays.

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[6] A comparison of the results of the microbiological method used here with other methods on reference proteins (7) suggests that the alanine values may be only two-thirds, approximately, of the true values.

## COMMUNICATIONS TO THE EDITOR

### The Niacin and Pantothenic Acid Content of Normal and Moldy Corn

DEAR SIR:

In our investigations of factors which affect the niacin and pantothenic acid content of corn hybrids it was found that immature corn (unpublished) had a higher content of these two vitamins than the same varieties when normally mature. Corn of high moisture content, irrespective of whether it is mature or immature, when stored may become moldy. The appearance of mold in both cases is a frequent occurrence under ordinary storage conditions.

In order to obtain more detailed information on the variation in the niacin and pantothenic acid content of normal and moldy corn, ten samples of apparently normal corn and thirteen samples of visibly moldy corn of the same varieties and grown in different locations were assayed for these vitamins by the microbiological method previously described (1). There was no way of knowing at that time whether the moldy corn was more immature than the apparently normal corn or vice versa. However, more recent results indicate that both groups of samples were mature but the moldy sample had a higher moisture content at some time or they would not have become moldy. The niacin content of the normal (non-moldy) corn varied from 19.7 to 31.2 (mean 24.1) $\mu$g/g, while the moldy samples varied from 21.2 to 37.7 (mean 30.00) $\mu$g/g. The results for pantothenic acid varied from 3.4 to 6.3 (mean 4.8) $\mu$g/g for normal (non-moldy) corn, while the moldy corn varied from 4.1 to 7.8 (mean 5.9) $\mu$g/g. These results are based on an air dried moisture content of about 10%. Where the samples were covered with a heavy mold the variation between normal and moldy corn, as described, was as much as 15 $\mu$g/g of niacin and 3.0 $\mu$g/g of pantothenic acid.

In order to obtain further information, samples of known mature corn were divided into two parts. One part was assayed as found. The moisture content of the other part was sufficiently increased and then incubated in covered enamel pans at room temperature (23°–25° C.) for about three days. The seeding of the samples was from the normal spores of the air. The moldy samples were then air dried to about 10% of moisture. The results for niacin and pantothenic acid on an air-dry basis were as follows:

| *No.* | *Sample* | *Niacin* μg/g | *Pantothenic acid* μg/g |
|---|---|---|---|
| 1 | Normal corn | 25.8 | 5.7 |
| 1A | Same moldy | 50.3 | 9.5 |
| 2 | Normal moldy | 27.0 | 5.6 |
| 2A | Same moldy | 52.8 | 7.8 |

These results show that when mold was artificially induced on normal corn it contained significantly higher niacin and pantothenic acid content than normal corn samples, indicating that the mold was at least part of the factor or factors involved in the above results.

Several investigators (2, 3) have shown that various fungi or microorganisms synthesize niacin and pantothenic acid and the above results appear to confirm these. However, we wished to obtain additional information on the synthesis of niacin and pantothenic acid by growing mold on a vitamin free medium. *Rhizopus* sp.* was isolated aseptically from the inside of the grain of moldy corn and grown in cotton-plugged erlenmeyer flasks containing the sterile vitamin-free medium. The medium contained 10 g. potassium nitrate, 5 g. potassium dihydrogen phosphate, 2.5 g. magnesium sulfate, 50 g. sucrose, and 2.0 g. vitamin-free casein hydrolysate per l. The incubation was for a period of about 3–4 days, when a sufficient amount of mycelia were obtained. These were collected, dried, and assayed for niacin and pantothenic acid with the following results, expressed on an air-dry basis:

| *Sample no.* | *Niacin* μg/g | *Pantothenic acid* μg/g |
|---|---|---|
| 1 | 159.0 | 74.8 |
| 2 | 154.7 | 74.7 |

These data show that this particular mold has the ability to synthesize niacin and pantothenic acid to a high degree. These findings offer an explanation for the increased niacin and pantothenic acid content of moldy corn over and above that of normal (non-moldy) corn.

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February 27, 1950

* Several species of *Rhizopus* were isolated from the moldy corn. The particular species used in this work was found to conform to the description of *R. nigricans* as given by Gilman (4) with respect to habit of growth and size of sporangia and spores.

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## Preparation of "Gluten" from Barley and Rye

SIR:

It is well known that, when a wheat-flour dough is manipulated under a stream of water, the starch is washed away and a cohesive, visco-elastic mass called gluten is obtained. This product contains about 80% of protein (N × 5.7), together with some carbohydrates, lipids, etc. Lusena (Cereal Chem. **27**: 167–178 (1950)) has recently shown that a similar product can be prepared from wheat flour by dispersing the protein complex in 0.005 *N* acetic acid, removing the starch, etc., by centrifuging, and reprecipitating the gluten by careful neutralization.

Heretofore, no products similar to wheat gluten have been prepared from any other cereal grain by ordinary washing; nor have we been able to prepare them by the Lusena method. But, after prior water extraction, and by using 0.01 *N* formic acid rather than acetic acid, "glutens" have now been prepared from both barley and rye flour. By comparison with wheat gluten, these new products are tougher, and less elastic, cohesive, and sticky. Mechanical working tends to disintegrate them. They are reminiscent of glutens prepared from low-protein flour of inferior quality. By the same procedure, oat flour has as yet yielded only a clay-like product with no elastic properties.

A comparative study of the chemical and physical properties of these "glutens," and of the relations of these properties to extraction methods, is in progress. It may well yield results of value in elucidating the basic structure of the protein components of bread dough.

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March 18, 1950

## BOOK REVIEWS

**Advances in Agronomy.** Vol. I. Edited by A. G. Norman. 439 pp. Academic Press Inc. New York, New York. 1949. Price $7.50.

The volume contains 10 chapters, each dealing with some phase of agronomic research or practice and each prepared by a recognized authority or authorities. Soil-crop relationships are emphasized throughout. Of the 10 chapters, six deal with soil in relation to plant growth and four with crop production. Each chapter includes a list of references which brings the subject matter up-to-date. These lists will provide a convenient source of reference material.

Hayward and Wadleigh of the U. S. Regional Salinity and Rebedoux Laboratory, Riverside, California, deal with saline and alkali soil-plant relationship in a thorough and comprehensive manner. Saline and alkali soils are differentiated and the physiological basis of salt and alkali tolerance of plants discussed. Specificity in salt tolerance for various species and varieties of crop plants are considered in some detail.

The second chapter on new fertilizers and fertilizer practices was written by Jones and Rogers of the T.V.A., Knoxville, Tennessee, who are in an excellent position to deal with the subject. Major attention is given to the development of new fertilizer materials and improved fertilizer practices. The chapter will be of interest not only to agronomists but to the Industry as well.

Martin G. Weiss of Iowa State College, Ames, authors a chapter on soybeans. The treatment of the subject is comprehensive in that it deals with all phases of the crop, including production, utilization, culture, and improvement by breeding and testing. The crop has several important effects upon the soil and, at the same time, the soil is an important factor in the production of the crop. Disease and insect controls are discussed.

A fourth chapter on the clay minerals of soil by J. E. Gieseking, University of Illinois, Urbana, deals both with mineralogical and plant relationships of this rather intricate subject. The article is exceptionally well done and will be of interest to soil scientists and those agronomists interested in fertility and plant nutrition problems.

Wm. J. White prepared the chapter on alfalfa improvement. This is a very informative article and presents the matter of seed setting and production in a clear and interesting fashion. His discussion of the not too well understood question of pollination and pollinating insects is worth careful reading. The author emphasizes the importance of breeding disease resistant varieties.

The chapter on Soil Micro-organisms and Plant Roots by Francis E. Clark, U.S.D.A. and Iowa Agricultural Experimental Station develops the interesting relationship existing between plants roots and soil microorganisms. The approach to the subject is one not frequently seen and for this reason is deserving of a careful reading.

A. S. Crafts and W. A. Harvey, University of California, deal with weed control. They discuss conventional methods as well as the newer methods of chemical control. In the latter they deal with a wide variety of materials and their herbicidal action. Their thorough treatment of the subject will be of interest both to agronomists and the manufacturers of weed killers.

The chapter on Boron in Soils and Crops by K. C. Berger, University of Wisconsin, describes methods of determination of the element in both soils and crops, the availability of soil boron and the boron requirements of crop plants. It includes an up-to-date review of the literature on the subject.

Ora Smith, Cornell University, has prepared a most excellent chapter on potato production. It includes a discussion of the improvement of the crop by breeding, the control of weeds, the requirements of the crop for nutrients, and methods of soil management most adaptable to the production of the crop. Included also are discussions of disease and insect control. This chapter should be of much interest to potato growers.

The subject of phosphorus fixation in soils is discussed by L. A. Dean, U. S. Department of Agriculture. Many factors are known to be involved in phosphorus fixation and an extraordinarily large amount of research has been devoted to the problem. The author has done an excellent job of bringing these researches together and summarizing them.

The volume as a whole is extremely well done and should be of wide interest to soil scientists, agronomists, manufacturers, and growers. The general excellence of Vol. I presages well for Vol. II which is to appear late in the year.

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**Chemical Engineers' Handbook.** Edited by John H. Perry. XV and 1942 pp. McGraw Hill Book Company Inc., New York. Third ed. 1950. Price $15.00.

In preparing the third edition of this standard reference work, the first and second editions of which were published in 1934 and 1941 respectively, the editor was assisted by an advisory board of 15 chemical engineers and 141 contributors comprising specialists in various phases of chemical engineering.

In the preface to the current edition, the editor points out that the normal peacetime rate of progress in the theory and practice of chemical engineering has been so great during the prewar, war, and postwar years that it was necessary to rewrite most of the major sections of the handbook.

The book contains sections covering the following topics: mathematical tables and weights and measures; mathematics; physical and chemical data; physical and chemical principles; flow of fluids; heat transmission; evaporation; general theory of diffusional operations; distillation and sublimation; gas absorption; solvent extraction and dialysis; humidification, dehumidification, and cooling towers and spray ponds; drying; adsorption; mechanical separations; size reduction and size enlargement; mixing of material; high-pressure technique; process control; movement and storage of materials; materials of construction; fuels; furnace and kilns; power generation and mechanical power transmission; refrigeration; plant location; electricity and electrical engineering; electrochemistry; accounting and cost finding; and safety and fire protection.

The new material which has been added in the present edition includes general theory of diffusional operations; furnaces and kilns; size enlargement; azeotropic distillation; multi-component distillation; extractive distillation; molecular distillation; and dialysis while the following chapters have been deleted: reports and report writing; indicators, qualitative analysis; and organic chemistry. This edition is printed in a larger page size (approximately 7 by 10 inches) which not only permitted the thickness of the book to be reduced but also allowed the use of larger sizes of graphs and illustrations which has increased the clarity. The book is printed on good paper, is well indexed and is remarkably free from typographical errors.

The edition provides a dependable source of information on principles, data, and practice in the various branches of chemical engineering and will be of inestimable value to cereal chemists, particularly those concerned with process development and control.

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# Cereal Chemistry

## EDITORIAL POLICY

*Cereal Chemistry* publishes scientific papers dealing with raw materials, processes, or products of the cereal industries, or with analytical procedures, technological tests, or fundamental research, related thereto. Papers must be based on original investigations, not previously described elsewhere, which make a definite contribution to existing knowledge.

*Cereal Chemistry* gives preference to suitable papers presented at the Annual Meeting of the American Association of Cereal Chemists, or submitted directly by members of the Association. When space permits, papers are accepted from other scientists throughout the world.

The papers must be written in English and must be clear, concise, and styled for *Cereal Chemistry*.

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## SUGGESTIONS TO AUTHORS

**General.** From January 1, 1948, an abstract will be printed at the beginning of each paper instead of a summary at the end, references will be numbered to provide the option of citing by number only, and date of receipt, author's connections, etc., will be shown in footnotes. Except on these points, authors will find the last volume of *Cereal Chemistry* a useful guide to acceptable arrangements and styling of papers. "On Writing Scientific Papers for Cereal Chemistry" (*Trans. Am. Assoc. Cereal Chem.* **6**: 1–22. 1948) amplifies the following notes.

Authors should submit two copies of the manuscript, typed double spaced with wide margins on 8½ by 11 inch white paper, and all original drawings or photographs for figures. If possible, one set of photographs of figures should also be submitted. Originals can then be held to prevent damage, and the photographs can be sent to reviewers.

**Titles and Footnotes.** Titles should be specific, but should be kept short by deleting unnecessary words. The title footnote shows "Manuscript received . . ." and the name and address of the author's institution. Author footnotes, showing position and connections, are desirable although not obligatory.

**Abstract.** A concise abstract of about 200 words follows title and authors. It should state the principal results and conclusions, and should contain, largely by inference, adequate information on the scope and design of the investigation.

**Literature.** In general, only recent papers need be listed, and these can often be cited more advantageously throughout the text than in the introduction. Long introductory reviews should be avoided, especially when a recent review in another paper or in a monograph can be cited instead.

References are arranged and numbered in alphabetical order of authors' names and show author, title, journal, volume, first and last pages, and year. The list is given at the end of the paper. Reference numbers must invariably be cited in the text, but authors' names and year may be cited also. Abbreviations for the names of journals follow the list given in *Chemical Abstracts* **40**: I–CCIX. 1946.

**Organization.** The standard organization involves main sections for abstract, introduction, materials, methods, results, discussion, acknowledgments, and literature cited. Alternately, a group of related studies, each made with different materials or methods, may require a separate section for each study, with subsections for materials and methods, and for results, under each section. Center headings are used for main sections and italicized run-in headings for subsections, and headings should be restricted to these two types only.

**Tables.** Data should be arranged to facilitate the comparisons readers must make. Tables should be kept small by breaking up large ones if this is feasible. Only about eight columns of tabular matter can be printed across the page. Authors should omit all unessential data such as laboratory numbers, columns of data that show no significant variation, and any data not discussed in the text. A text reference can frequently be substituted for columns containing only a few data. The number of significant figures should be minimized. Box and side heading should be kept short by abbreviating freely; unorthodox abbreviations may be explained in footnotes, but unnecessary footnotes should be avoided. Leader tables without a number, main heading, or ruled lines are often useful for small groups of data.

Tables should be typed on separate pages at the end of the manuscript, and their position should be indicated to the printer by typing "(TABLE I)" in the appropriate place between lines of the text. (Figures are treated in the same way.)

**Figures.** If possible, all line drawings should be made by a competent draftsman. Traditional layouts should be followed: the horizontal axis should be used for the independent variable; curves should be drawn heaviest, axes or frame intermediate, and the grid lines lightest; and experimental points should be shown. Labels are preferable to legends. Authors should avoid identification in cut-lines to be printed below the figure, especially if symbols are used that cannot readily be set in type.

All drawings should be made about two to three times eventual reduced size with India ink on white paper, tracing linen, or *blue*-lined graph paper; with any other color, the unsightly mass of small grid lines is reproduced in the cut. Lettering should be done with a guide using India ink; and letters should be $\frac{1}{16}$ to $\frac{1}{8}$th inch high after reduction.

For difficult photographs, a professional should be hired or aid obtained from a good amateur. The subject should be lighted to show details. A bright print with considerable contrast reproduces best, and all prints should be made on glossy paper.

All original figures should be submitted with one set of photographic reproductions for reviewers, and each item should be identified by lightly writing number, author, and title on the back. Cut-lines (legends) should be typed on a separate sheet at the end of the manuscript. "Preparation of Illustrations and Tables" (*Trans. Am. Assoc. Cereal Chem.* 3: 69–104. 1945) amplifies these notes.

**Text.** Clarity and conciseness are the prime essentials of a good scientific style. Proper grouping of related information and thoughts within paragraphs, selection of logical sequences for paragraphs and for sentences within paragraphs, and a skillful use of headings and topic sentences are the greatest aids to clarity. Clear phrasing is simplified by writing short sentences, using direct statements and active verbs, and preferring the concrete to the abstract, the specific to the general, and the definite to the vague. Trite circumlocutions and useless modifiers are the main causes of verbosity; they should be removed by repeated editing of drafts.

**Editorial Style.** A.A.C.C. publications are edited in accordance with *A Manual of Style*, University of Chicago Press, and *Webster's Dictionary*. A few points which authors often treat wrongly are listed below:

Use names, not formulas, for text references to chemical compounds. Use plural verbs with quantities (6.9 g. were). Figures are used before unit abbreviations (3 ml.), and % rather than "per cent" is used following figures. All units are abbreviated and followed by periods, except units of time, which are spelled out. Repeat the degree sign (5°–10° C.). Place 0 before the decimal point for correlation coefficients ($r = 0.95$). Use * to mark statistics that exceed the 5% level and ** for those that exceed the 1% level; footnotes explaining this convention are no longer required. Type fractions on one line if possible, e.g., $A/(B + C)$. Use lower case for farinograph, mixogram, etc., unless used with a proper name, i.e., Brabender Farinograph. When in doubt about a point that occurs frequently, consult the Style Manual or the Dictionary.

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## Fleischmann's Yeast— *for finest fermentation*

Constant improvement over the years has made today's Fleischmann's Yeast the finest that bakers have ever used.

Reasons for this are many. Each pure yeast cell isolated by the Fleischmann Laboratories' technicians is "babied" in the Fleischmann plants as it's grown and developed into thousands and thousands of pounds of baker's yeast.

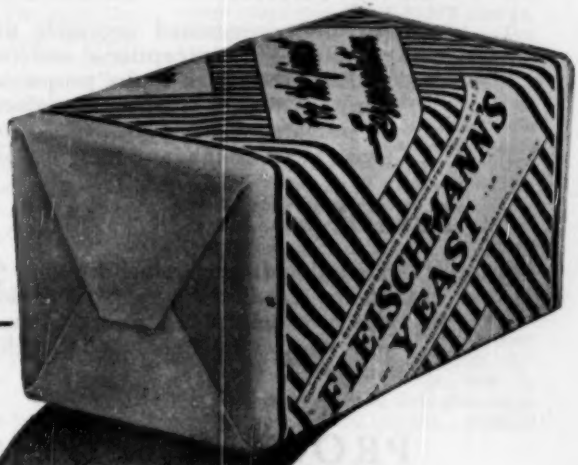
During manufacture, for instance, the yeast is repeatedly washed in pure water to safeguard its uniformity. In the Fleischmann cutting and wrapping rooms, even the *air* is filtered to assure yeast purity. *After* manufacture, the yeast is checked at the plant and later in various Fleischmann control laboratories.

It is such endless, constant improvement and checking that gives you yeast that provides balanced fermentation . . . that "conditions" the dough batch throughout the entire fermentation stage. This helps assure *good volume, grain,* and *texture* in the finished baked goods.

In 1950, as for over 80 years, every facility and service of the makers of Fleischmann's Yeast will again be devoted constantly towards safeguarding

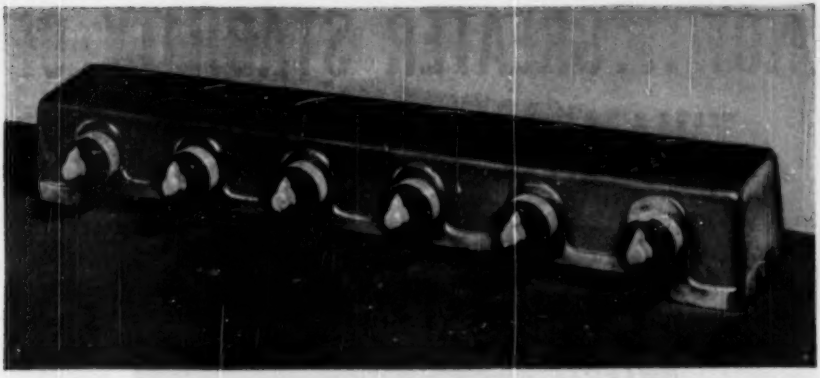
**fermentation — your business and our business**

*In the new, bright blue-and-white striped wrapper*——




# Yeast

*— always "busy" in the dough...*
***NEVER FLASHY...NEVER SLOW!***



# SARGENT HOT PLATE

## *Evaporations, Digestions*
## *Crude Fiber Determinations*
## *Extractions With Soxhlet Apparatus*

The six-place Sargent Hot Plate is tapped to accommodate seven support rods ½"x27" for use with Crude Fiber and Soxhlet assemblies. The solid disc heaters are individually controlled by separate switches (3 heat). Cool switching is assured, for the heaters are mounted on porcelain spools and have no direct contact with the base. The perforated bottom baffle plate acts as a heat reflector, eliminating scorching under the hot plate. The base itself is of cast aluminum. Dimensions: length, 28½"; width, 5¾"; height, 4".

**S-41315** HOT PLATE — Electric, Sargent. For 115 volt AC/DC circuits. .....**$85.00**

**S-31870** CRUDE FIBER ASSEMBLY —Six-Place, Electric, Sargent. Consisting of one **S-41315** Hot Plate; seven rods of stainless steel, ½" x 27" with 3/8-16 thread; six **S-4705** beakers, high form, without pourout, 600 ml., Pyrex; six **S-22742** condensers, reflexed hemisphere, Sargent; two **S-31872** clamps, water connector; five **S-31873** clamps, condenser support; rubber tubing. For 115 volt AC/DC circuits.....**$125.00**

**S-31765** EXTRACTION ASSEMBLY —Six-Place, Large, Electric, Sargent. For medium and large Soxhlet assemblies, consisting of one **S-41315** Hot Plate; six rods of stainless steel, ½"x27" with ⅜" thread; 12 **S-31769** clamps, size B, spring, rubber covered. Less glassware. For 115 volt AC/DC circuits .....**$120.00**

**S-31767** EXTRACTION ASSEMBLY —Six-Place, Small, Electric, Sargent. For small Soxhlet assemblies, consisting of one **S-41315** Hot Plate; six rods of stainless steel, ½" x 27" with ⅜" thread; 12 **S-31769** clamps, size A, spring, rubber covered. Less glassware. For 115 volt AC/DC circuits....**$120.00**

# SARGENT

*SCIENTIFIC LABORATORY INSTRUMENTS · APPARATUS · CHEMICALS*

**E. H. SARGENT & COMPANY, 4647 W. FOSTER AVE., CHICAGO 30, ILLINOIS**
MICHIGAN DIVISION, 1959 EAST JEFFERSON STREET, DETROIT 7, MICHIGAN
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# NOW...GREATER STABILITY OF THIAMINE IN FLOUR WITH

## *Thiamine Mononitrate Merck*

CEREAL chemists have welcomed with enthusiasm the introduction of Thiamine Mononitrate, now available in the full line of Merck Vitamin Mixtures for Flour Enrichment.

*Thiamine Mononitrate is a more stable form of thiamine, assures a more complete retention of vitamin $B_1$ content in enriched flour—even under adverse conditions of temperature and humidity during shipping and storage.*

Leading cereal chemistry laboratories participated in the thorough investigation of Thiamine Mononitrate in enriched flour. Since then, extensive commercial experience and rigorous field tests have confirmed that Thiamine Mononitrate represents a signal improvement in flour enrichment; that it offers a degree of protection hitherto not available.


Each ounce contains 369 milligrams Thiamine Mononitrate

**NO CHANGE IN LABELING REQUIRED**

Thiamine Mononitrate meets every requirement for thiamine under the Federal Standards of Identity for enriched flour. No need for change in the labeling on bags or packages to comply with Federal requirements when you use these improved Merck Vitamin Mixtures.


Each ounce contains 738 milligrams Thiamine Mononitrate

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*Manufacturing Chemists*
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## MERCK ENRICHMENT MIXTURES

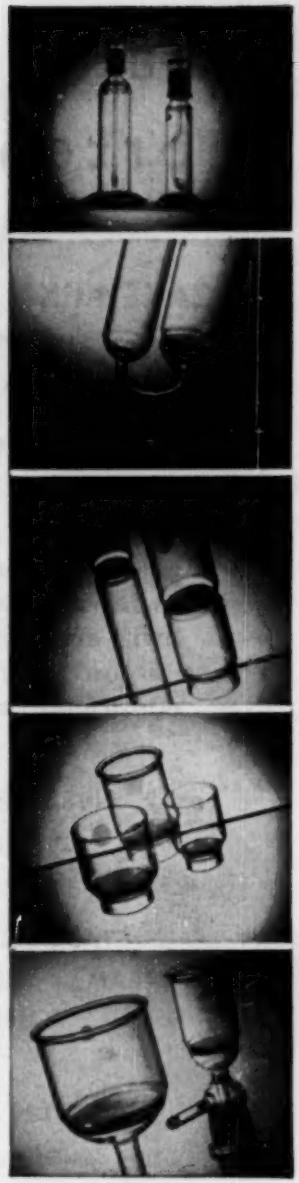
# Corning Announces New Tubular Fritted Glass Filters

A new tool for filtration or gas dispersion, Pyrex brand Tubular Fritted Glass Filters offer you many important advantages. Suitable for either pressure or vacuum applications, they can be obtained in accurately controlled ultra-fine, fine, medium and coarse porosity.* The connection is corrugated to accommodate several sizes of rubber tubing, making setups easy. Inspection for cleanliness is simplified by the clear bottom.

Made entirely of Pyrex brand glass No. 7740, Corning Tubular Fritted Glass Filters prevent contamination, eliminate absorption of active ingredients. Fired to uniform hardness, you have no trouble with particles sloughing off. They are available in three sizes: 5/8" diameter by 2", 5/8" by 4" and 1" by 8"—dimensions of effective outside fritted section.

For all-around filtration, gas washing and gas absorption, Corning's complete line of Pyrex brand Fritted Glassware will answer most of your requirements. It is available in a wide variety of standard shapes and sizes and in five porosities. For accurate analysis and long service life specify Pyrex brand Fritted Glassware. Your laboratory dealer stocks it.

*For technical information regarding flow rates and pore sizes of Fritted Glassware, write for Bulletin B-80.



**CORNING GLASS WORKS** PYREX **CORNING, N. Y.**

*Corning means research in Glass*

*Technical Products Division:* Laboratory Glassware, Glass Pipe, Plant Equipment, Lightingware, Signalware, Gauge Glasses, Optical Glass, Glass Components

# What's your particular shortening problem?

**CAKE MIX? PIE CRUST MIX?**
**COFFEE CAKE MIX?**
**DOUGHNUT MIX? COOKIE MIX?**
**UNUSUAL CLIMATIC CONDITIONS?**

● No matter what your problem may be Swift can help you solve it. There's a Swift *shortening for every use.* Standardize on *Swift's* for uniform results . . . controlled performance.

INVESTIGATE SWIFT'S *COMPLETE*
SHORTENING SERVICE

STABILIZED LARDS VEGETABLE OILS
VREAM VREAMAY XXX VREAM TRI-CO
FLUFF DRIED EGG ALBUMEN

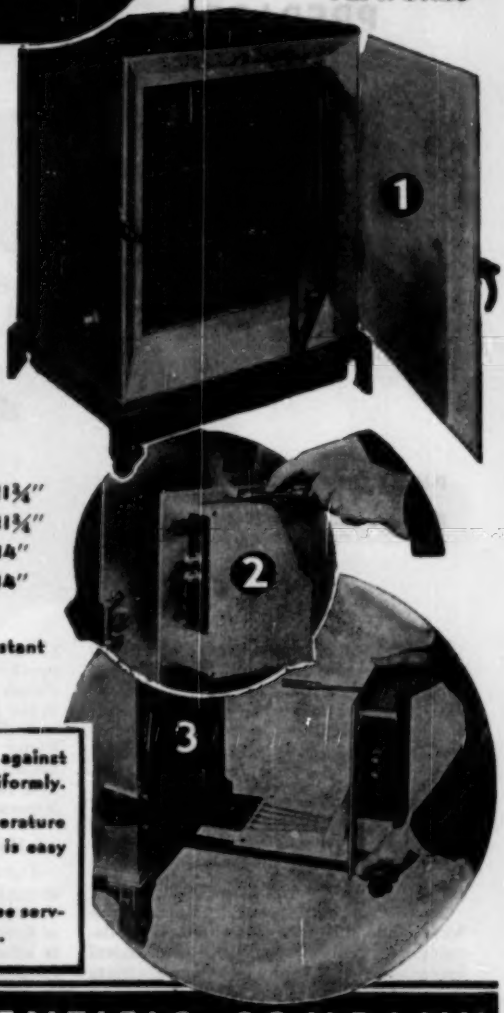
*Let Swift's Technicians assist*
*with your Prepared Mix Problems*

## SWIFT & COMPANY

*Chicago 9, Illinois*

# CENCO-DE KHOTINSKY OVENS

**NOTE THESE 3 FEATURES**




**Cenco-DeKhotinsky Ovens are constructed with three walls; two of transite and one of monel metal. The asbestos-magnesia packing between the outer transite walls serves as effective insulation. The monel metal inner wall is provided with ports located properly for uniform distribution of hot air.**

**95100A for 115 volts—14¾"x12"x11¾"**
**95100B for 230 volts—14¾"x12"x11¾"**
**95105A for 115 volts—19¾"x17"x14"**
**95105B for 230 volts—19¾"x17"x14"**

**• Write for Descriptive Bulletin 5, Constant Temperature Equipment.**

1. **Triple-wall construction guards against heat loss—distributes heat uniformly.**
2. **Single tray mounting for temperature control and heating elements is easy to remove.**
3. **Proved for long and trouble-free service in hundreds of laboratories.**

CENTRAL SCIENTIFIC COMPANY

*Scientific* CENCO *Apparatus*

700 IRVING PARK ROAD CHICAGO

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# A HELPING HAND FOR MAKERS OF PREPARED MIXES

**PHOSPHATES FOR LEAVENING**

One of the most important problems in the manufacture of prepared mixes is that of selecting the most effective leavening. For upon the leavening largely depends the volume, lightness, texture and color of the baked product as well as the shelf life of the mix.

This problem is somewhat involved because leavening ideally suited for one type of mix may not be as efficient for another. Therefore, the leavening must be tailor-made for each individual mix.

From Victor's Research Laboratories have come many of the outstanding developments in the science of chemical leavening. V-90 (anhydrous monocalcium phosphate) and Victor Cream (sodium acid pyrophosphate) are two such leavening chemicals that have gained wide acceptance in the prepared mix industry. Used alone and often in combination with each other, Victor Phosphates for leavening have helped improve many prepared mix products resulting in brand establishment and increased sales for manufacturers.

### A Helping Hand for Product Problems

For 50 years Victor Chemical Works has specialized in chemicals for the food industry. The wide experience of our staff of research chemists and chemical engineers is offered as a helping hand to the food industry.

VICTOR CHEMICAL WORKS

**VICTOR CHEMICAL WORKS**

WORLD'S LARGEST MANUFACTURERS OF FOOD PHOSPHATES

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# Service When It Counts

Every so often, we all need assistance and need it badly, to furnish the answers to many of our problems. In the Flour and Cereal Industry, N-A's Flour Service Division has been supplying practical, down-to-earth solutions to many of the industry's problems for over 25 years. Using its broad background of experience in the manufacture of time-tested products for maturing, bleaching, and enriching, the N-A Flour Service Division is ready to put its nation-wide service organization and extensive laboratory and research facilities at your service. They will work closely with your own staff and consultants to produce a concrete, individual solution to fit your particular needs.

For more details, call your nearest N-A Representative today and find out how this service can work profitably for you.

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